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Glare Reduction by Dark Facial Markings and Bills in Birds

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GLARE REDUCTION BY DARK FACIAL MARKINGS AND BILLS IN BIRDS

By

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GLARE REDUCTION BY DARK FACIAL MARKINGS AND BILLS IN BIRDS

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ABSTRACT

Avian facial plumage, bill coloration, and feather microstructure may serve one or more adaptive functions. Several researchers have proposed that dark eyestripes, bills, and facial masks aid in reducing glare, however, there have been relatively few tests of this hypothesis. Dark facial markings have been shown to have an adaptive glare-reduction function in recent field studies of a few species, but this hypothesis has never been tested in a broad multispecies analysis. It is likely that feather microstructure influences feather brightness and has an effect on the efficacy of glare reduction properties of feathers. I examined the link between dark facial markings and glare reduction under natural lighting conditions in several bird species, using a spectrometer probe placed in the eye-position of museum specimens. As a measure of glare, I quantified the reduction in irradiance in full, natural sunlight, for specimens varying in bill and head plumage coloration and pattern. Each specimen was tested with the head held at various angles to mimic natural foraging positions. I also quantified the brightness of bills and plumage surrounding the eye of these same specimens using reflectance spectroscopy. Correlations between irradiance measurements and the bill and plumage brightness were analyzed. Facial feather microstructure, proximal and distal barbule density, and pith:cortex ratio were examined using scanning electron microscopy. I then correlated these characteristics to plumage brightness of both light and dark patches.

A significant relationship with average head darkness and reduction in irradiance values was found when the eye faced directly into the sun, and when it was rotated horizontally 45° away from the sun. Dark patches in the anterior and posterior dorsal quadrants are most important in this reduction in irradiance. Of feather microstructural features, the pith:cortex ratio affected plumage brightness of the entire head, with a larger pith:cortex ratio being associated with darker plumage. Proximal and distal barbule density also play a role in feather brightness. Increased proximal barbule density was correlated with darker plumage, while in an opposing trend increased distal barbule density was correlated with lighter plumage. Future research could expand on the link between these and related features to plumage coloration, with an emphasis on glare reduction or their functions in the feathers of diurnal species.

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CHAPTER I: INTRODUCTION

Dark facial markings might serve one or more adaptive functions that can vary among species. In mammals, the purpose of dark facial markings in carnivores and herbivores can range from crypsis to social signaling (Ortolani 1999). Glare reduction has been proposed as one of the reasons for increasing amounts of dark coloration surrounding primate eyes in areas closer to the equator (Santana et al. 2012; Diogo and Santana 2017) and for dark eye markings in crepuscular species (Ortolani 1999). For snakes, different explanations have been proposed, including sight-lines to target prey and signaling conspecifics (Lillywhite and Henderson 1993; Kwiatkowski and Burt 2011). The diverse functions of dark facial markings in birds can include glare reduction in sunny habitats, sight-lines for foraging on rapidly moving prey, and sexually selected signals (Burtt 1986; Caro 2005; Galván and Sanz 2009; van Dijk et al. 2010; Yosef et al. 2012).

Hypotheses for Adaptive Functions of Dark Plumage and Bills

Glare Reduction

Glare from sunlight can be a major hindrance to birds, especially for those foraging in sunny habitats, possibly impacting the success of foraging or

communication attempts (Martin and Katzir 2000; Théry 2006; Fernández-Juricic and Tran 2007; Fernández-Juricic et al. 2012; Beauchamp 2017). The type of glare that may interfere with bird vision is called disability glare; wherein excess light enters the bird's eye or the image of the sun is perceived by the retina (Martin and Katzir 2000; Fernández-Juricic et al. 2012). This excess light scatters within the eye chamber and reduces the eye's ability to discern low contrast objects and makes sharp resolution of targets difficult (Martin 2007; Fernández-Juricic et al. 2012). Glare can be problematic for both species trying to avoid predators (Carr and Lima 2014; van den Hout and Martin 2011) or predators scanning for prey (Yosef et al. 2012). It has also been proposed that birds with larger eyes may struggle more with disability glare (Martin and Katzir 2000). A bird's eye is similar to a human's in that light enters the eye, undergoes refraction from the cornea and lens, and this refracted light forms an image at the retinal lining at the back of the eye (Hall and Ross 2006). Visual fields vary between species, as some birds have forward facing eyes, such as owls, or have eyes further to side, such as raptors or waterbirds (Martin 2007). This differing placement of the eyes for each species can affect its binocular vision, for example some raptor eyes lack binocular overlap under the bill, but have the ability to see more towards the back of the head (Martin 2007). Unlike humans many birds also possess a heavily pigmented eye structure called a pecten with provides nutrients to the eye and creates a blind spot in the visual field of each eye (Martin 2007). Some researchers have proposed the pecten may also serve

to reduce incident light or glare from the sun in the eye (Barlow and Ostwald 1972; van den Hout and Martin 2011; Brown 2017). It has also been shown birds have the ability to detect polarized light, double cones have been suggested to be polarized light receptors for birds, but the mechanics behind it are not well understood (Kreithen and Keeton 1974; Muheim 2011). Sensitivity to polarized light has also been found in some fish and invertebrates, and further research into this topic for birds is ongoing (Muheim 2011).

Several researchers have proposed that dark eyestripes, facial masks, and bills aid in reducing glare in many species (Ficken and Wilmot 1968; Burtt 1984; Burtt 1986; Brooke 2010; Yosef et al. 2012; Diogo and Santana 2017), analogous to the use of dark smudges below the eyes of athletes to reduce disability glare (De Broff and Pahk 2003). Dark facial markings have been shown to have an adaptive glare-reduction function in field studies of a few bird species. Masked Shrikes (*Lanius nubicus*) have dark facial masks that help reduce glare while foraging (Yosef et al. 2012). Foraging Masked Shrikes were placed into three experimental groups. Birds with facial masks painted white changed their angle of attack away from the sun and experienced lower levels of foraging success compared to birds in control groups. Therefore, the dark facial masks of Masked Shrikes appear adaptive in reducing glare, allowing the birds to capitalize on the advantages of striking prey while flying towards the sun in open, desert habitats.

The most comprehensive study on dark facial markings related to the glare reduction hypothesis provided both laboratory and field data on the function of dark facial markings in species of New World warblers (Family Parulidae, Burtt 1986). Burtt (1986) examined the possible anti-glare properties of both facial markings and bills among members of this clade. Many of the warbler species examined have dark eyestripes, but field observations of foraging patterns suggest that dark bill color might play a larger role in glare reduction than dark facial markings. Birds with dark upper mandibles (the maxilla) were more likely to forage in sunny habitats, but no correlation was found for dark facial plumage (Burtt 1986). The lack of support for the function of dark eyelines or facial markings in reducing glare might have been attributable, in part, to how Burtt (1986) collected his field data wherein he did not account for bird sex. Sexual dichromatism exists in the facial patterns in many warbler species, with black eye lines seen in males of 40 % of species (twice what was expected based on Burtt's (1986) assumption that all colors had the same likelihood of appearing on these regions of the bird). In contrast, 12 % of species show these patterns in females, whose most common eye line color was gray (Burtt 1986).

Other Adaptive Hypotheses

Beyond glare reduction, multiple additional hypotheses have been proposed to explain dark eyestripes, other dark facial markings, and dark bills in birds. Some or all of these traits might function for discrimination of individuals in social species, as sexually selected signals, or to provide sight lines that increase

the efficiency of tracking and capturing fast moving prey. These varied hypotheses have been suggested by several researchers since the 1960's, but there have been few studies to test the adaptive significance of dark facial markings in these contexts (e.g., Burtt 1986; Ortolani 1999; Kwiatkowski and Burt 2011; Yosef et al. 2012).

Sexually selected trait-

Dark facial markings carry social significance in certain species, with the markings indicating a bird's social status and impacting its mating and feeding opportunities (Hill 1987; Ferns and Hinsely 2004; Dunn et al. 2008; Galván and Sanz 2009). The social connotations of plumage or color patches varies widely but their role in signaling status is clearly important in some species. For Great Tits (*Parus major*), white cheek patches function as social signals to conspecifics about individual quality (Ferns and Hinsely 2004). Both male and female individuals gained advantages when the black borders of their cheek patches had increased border uniformity (Ferns and Hinsely 2004). Great tits with the cheek patches made artificially uneven were more frequently denied access to bird feeders by conspecifics (Galván and Sanz 2009).

This pattern is also evident with dark eyelines or facial masks; a comprehensive plumage coloration analysis shows that males within Parulidae, on average, have darker colored eye lines (Burtt 1986). In the polygamous Eurasian Penduline Tit (*Remiz pendulinus*), males have larger eye-stripes than

females. Males with wider eyestripes are considered more attractive, but are also more likely to abandon their nests forcing heavier parenting costs upon the female (van Dijk et al. 2010). Similar patterns of mate choice and actions can also be seen in the Common Yellowthroat (*Geothlypis trichas*), where males with larger black facial masks are thought to be more attractive to females (Thusius et al. 2001; Mitchell et al. 2007).

Sight-lines-

Eyestripes could potentially serve as sight-lines for birds that catch swift moving prey by increasing their targeting accuracy (Ficken and Wilmot 1968). Aerial insectivores in North America show a high probability of having eye markings that could serve as sight lines that improve their precision when aiming at swift moving prey (Ficken et al. 1971). Many waterbirds, such as sandpipers and herons, also exhibit darker facial markings that consist of a stripe moving from the eye to beak (Ficken et al. 1971).

Objectives

Several adaptive functions might explain the evolution of dark eyestripes, facial masks and bill color; however, few studies have tested adaptive roles of dark facial markings and bills (e.g., Ficken and Wilmot 1968, Burtt 1984,1986; van Dijk et al. 2010; Yosef et al. 2012). This study conducts an examination of the glare reduction hypothesis by quantifying the degree of glare reduction in museum specimens representing numerous species with varying facial and bill

patterns under standardized lighting conditions. The following specific hypotheses and predictions were tested.

Glare Reduction Hypothesis:

H_0: Dark eyestripes, other facial markings, and bill color have no adaptive link to glare reduction.

H_1: Melanic eyestripes, other dark facial patterns surrounding the eye, and/or dark bills reduce glare. The predictions that supports H_1 are the following:

- Prediction 1: Birds with darker head plumage patches and darker bills will have less light reflected into their eyes.
- Prediction 2: Birds with a greater proportion of dark plumage near the eye will have less light reflected into their eyes.

MATERIALS AND METHODS

Morphometric and Spectrometer Data Collection

Specimens: Thirty-seven museum skins of thirty-three avian species representing a range of taxa and plumage colors and variation in dark facial markings and bill colorations were used to quantify levels of potential glare reduction (Appendix A). Three of these species are sexually dichromatic. Specimens were housed in the Stephen F. Austin State University museum collection. Specimens were identified as lacking or having dark facial masks (this includes birds with wholly dark heads like the American Crow (*Corvus brachyrhynchos*)), based on two independent observers using *The Sibley Guide to Birds, Second Edition* (Sibley 2014). Birds with wholly dark heads were included in the masked group as their overall dark plumage may serve as a large whole-head mask for glare reduction. No discrimination was made regarding breeding or not-breeding plumage, so both types of plumage are represented among the specimens.

Plumage brightness and patch size measurements: Plumage and bill brightness were measured for each specimen using reflectance spectroscopy, which measures the percentage of light reflected by a sample, and is expressed

as percent reflection (or brightness %) (Endler and Théry 1996; Gomez and Théry 2007; Santos et al. 2007; Armenta et al. 2008; McCoy et al. 2018). Black plumage can express reflectance measurements below 10% (McCoy et al. 2018). Meanwhile white plumage can be expressed as reflectance values as high as 50-70%. Measurements were taken using a USB 2000 (Ocean Optics) spectrometer and DH-mini deuterium-halogen lamp (Ocean Optics) following standard protocols (Gomez and Théry 2007; Santos et al. 2007). A bifurcated optic cable (R400-7-UV/VIS Ocean Optics) provided light from the lamp for illumination and a reflectance probe encased in a rubber stopper was held at 90° from the sample surface (Mennill et al. 2003). Prior to measuring samples baseline readings were established using a white standard (Spectralon Diffuse Reflectance Standard, Labsphere) and by shuttering the lamp (Stavenga and Wilts 2014). Reflectance data were collected with OceanView (Ocean Optics) software with 10 scans averaged across a single reading. Reflectance values (percentages) were calculated from the spectrum of 300-700 nm to represent the average avian visual spectrum (Pearn et al. 2003; Mays et al. 2006; Hofmann et al. 2007; Avilés et al. 2011; Pascual et al. 2014; Stavenga and Wilts 2014).

Plumage was categorized and examined in two ways for each specimen: distinct plumage patches, and with head regions divided into quarters with eye as center point. Utilizing both methods makes it possible to see if the natural shape of dark patches and/or the placement of dark coloration in specific locations around the eye affects glare. Patch and quarter reflectance was measured to

allow four methods of analysis relative to the potential effects of following regions to glare reduction: the entire head, a binary measurement of dark facial masks (present or absent), reflectance of individual patches based on proportion of area, and quarterly reflectance of the head with the eye as center point.

Patches: To quantify relative patch size on each museum specimen, a system modified from Mennill et al. (2003) and Crary and Rodewald (2012) was used. Each specimen was placed in a white box with a ruler lining the top edge next to the specimen's head and a digital photo was taken from 30 cm away (Mennill et al. 2003; Crary and Rodewald 2012). The camera flash was used as the light source and a single photo was taken of each specimen (Crary and Rodewald 2012). The background of each photo was removed and colors edited using the Auto Tone setting in Adobe Photoshop CS6 (v 13.0) (Vickrey et al. 2018). Head and patch sizes were quantified using the lasso tool (Tonra et al. 2014). The number of pixels for each patch was divided by the total number of pixels for the entire head to quantify a proportional size for each patch.

Each patch was numbered (Fig. 1). Three scans for reflectance values (reflectance %) were taken for each patch in each quarter (defined below) using the reflectance spectroscopy protocol and an average reflectance value was quantified for each patch. From these data a patch index was created to allow comparison of patch size and brightness across specimens. Patch index was calculated with the following formula: Patch Index = (Patch 1 brightness × Patch 1 Proportion of area) + (Patch 2 brightness × Patch 2 Proportion of area) +

(etc...). With this patch index it is possible a bird with large dark patches may have an equal patch index value to a bird with small bright patches, but it was felt this index was most representative of patch brightness.

Figure 1. Example of how the different plumages patches would be labeled on a museum specimen.

Quarters: The head of each specimen was divided into four quarters (Quarter 1: dorsal and anterior to the eye; Quarter 2: ventral and anterior to the eye; Quarter 3: ventral and posterior to the eye; Quarter 4: dorsal and posterior to the eye; Fig. 2) with the eye as center point. Each patch within a quarter underwent three scans for reflectance values, using the methods described above, and were averaged within each quarter. Reflectance values for each quarter were calculated by averaging the reflectance values of all patches in that quarter. The purpose of dividing the head into quadrats with the eye as center point is to address the question of if the location of dark plumage around the eye is associated with glare reduction across several species. Relevant angles of

irradiant light may be species specific, requiring a 360° 3D scan of the head for each species, so this method allows a more generalized analysis of this question. Testing this across different head tilts and angles is especially relevant as purposeful head tilting is observed in some species of shorebirds, and this action may be a deliberate attempt to reduce glare (van den Hout and Martin 2011).

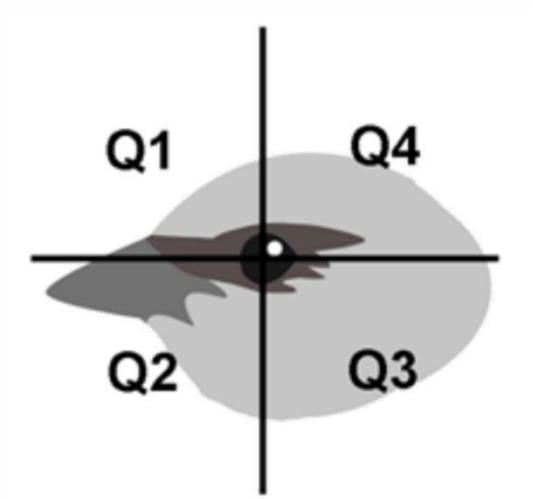


Figure 2. Representation of a specimen with regions divided into quarters for measuring patch reflectance. Every patch in a quarter had its reflectance measured and then an average reflectance from all patches was calculated for each quarter.

Glare measurements: To quantify potential light reduction a USB 2000 spectrometer (QP400-2-SR, Ocean Optics) with a cosine corrector (CC-3-UV-S, Ocean Optics) placed through the skull of the museum specimen and placed in the eye position on the opposite side of the head to mimic the bird's field of view (Fig. 3) (Svenmarker et al. 2011). The cosine corrector has a measurement face of 6.35 mm, can record wavelengths 200nm to 2500nm, and works as an optical diffuser that enables it to pick up light across a larger range of angles than other

probes (Ocean Optics; Mustafa et al. 2016). The spectrometer records ambient light (or brightness) as irradiance (µmol m^{-2} s^{-1}: micromoles per meter squared per second; Endler 1990, 1993; Altshuler 2003; Avilés et al. 2008, 2011; Zheng et al. 2008), and reduction in brightness is used here as an indirect measure of potential glare. The spectrometer reads this irradiance as photosynthetically active radiation (PAR), and measures the wavelengths 400 nm to 700 nm (Dye 2004). This unit of measure was chosen as it is often used as a measurement of ambient light in biological studies, and is relevant to both photosynthesis and vision (Endler 1990, 1993; Altshuler 2003; Avilés et al. 2008, 2011). Cosine correctors allow spectrometer probes to pick up irradiance on a 180° plane (Avilés et al. 2011), which will enable the probe to detect light bouncing off feathers surrounding a specimen's eye. The irradiance measurements were taken with the same spectrometer described above and analyzed with the software SpectraSuite (Ocean Optics) (Altshuler 2003; Avilés et al. 2011). Within SpectraSuite irradiance was measured under the absolute irradiance setting, with an average of 30 scans per reading (Théry 2001; Gamon et al. 2005).

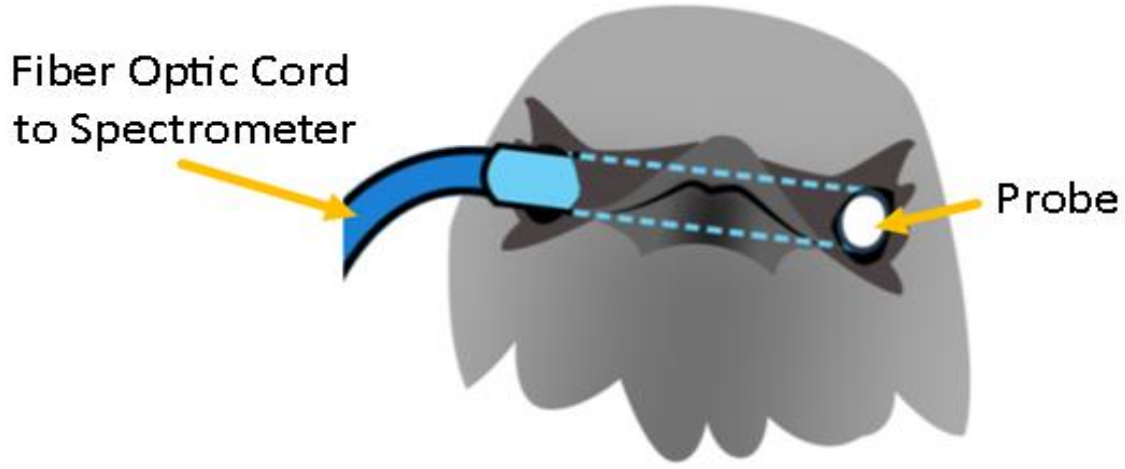


Figure 3. The cosine corrector or "probe" inserted through one side of the specimen's skull so that the probe was placed in the eye position on the other side of the skull.

Natural, unfiltered sunlight was used as the light source (Gehrmann 1987; Théry and Endler 2001). Irradiance measurements were only taken on sunny, cloudless days throughout the year between the hours of 1000 to 1400 (when the sun is highest in the sky, and the outside site selected for measurements had an unobstructed view of the sun) (Altshuler 2003; Carr and Lima 2014). The cosine corrector and fiber optic cable were threaded through a device that securely held both specimen and cosine corrector in place (Fig. 4). Before taking irradiance measurements of specimens, a daily baseline irradiance was gauged with the cosine corrector placed in the device at the same orientation as the first specimen to be measured. The device was oriented towards the sun with the use of a steel protractor and routinely reoriented as the sun's position changed during the survey hours, baseline irradiance was also rechecked at these times.

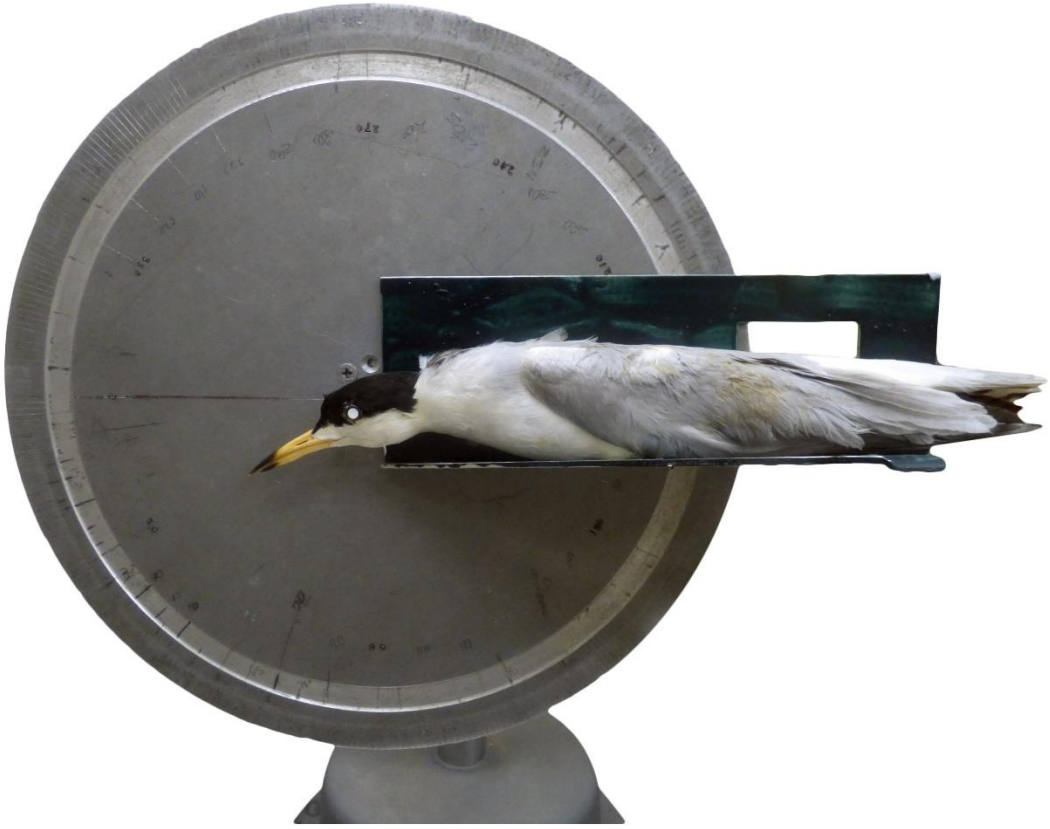


Figure 4. The device used for quantifying irradiance. The metal disc can rotate 360°. The spectrometer probe (the white dot centered on the eye of the specimen) runs through the disc, into the side of the specimen's skull, and into the eye socket on the other side.

The face of the instrument faced the sun so that the cosine corrector placed in the location of the bird's eye was pointing to the sun with no obstructions (Fig. 5). The first bird chosen for measurements also had mid-session and end-session measurements taken to test for potential changes in light levels. Once a specimen was secured to the instrument, an irradiance measurement was taken with the spectrometer. The specimen was then removed from the cosine corrector, making sure to keep the probe in the same

location and then a baseline measurement was taken. The difference between the measurements is expressed as the change in light or reduction in light percentage (Appendix B). This procedure was repeated three times for each bird with the head rotated on the horizontal plane at three different orientations to the sun (0°, 45°, and 90°). At the 45° and 90° angles the light was coming from the front of the head (Carl 1987; Brown 2017) (Fig. 6). Within each of these rotations the head was also tilted at three angles (135°, with head up; 90°; and 45°, with head down) along the sagittal or longitudinal plane (Fig. 5). These three angles were chosen as a baseline to represent typical head positions a bird might take while foraging. All measurements for each specimen were taken within a few minutes of each other. Three separate, complete daily replications were performed for most birds at each head tilt (0° N = 30, 45° N = 37, 90° N = 36).

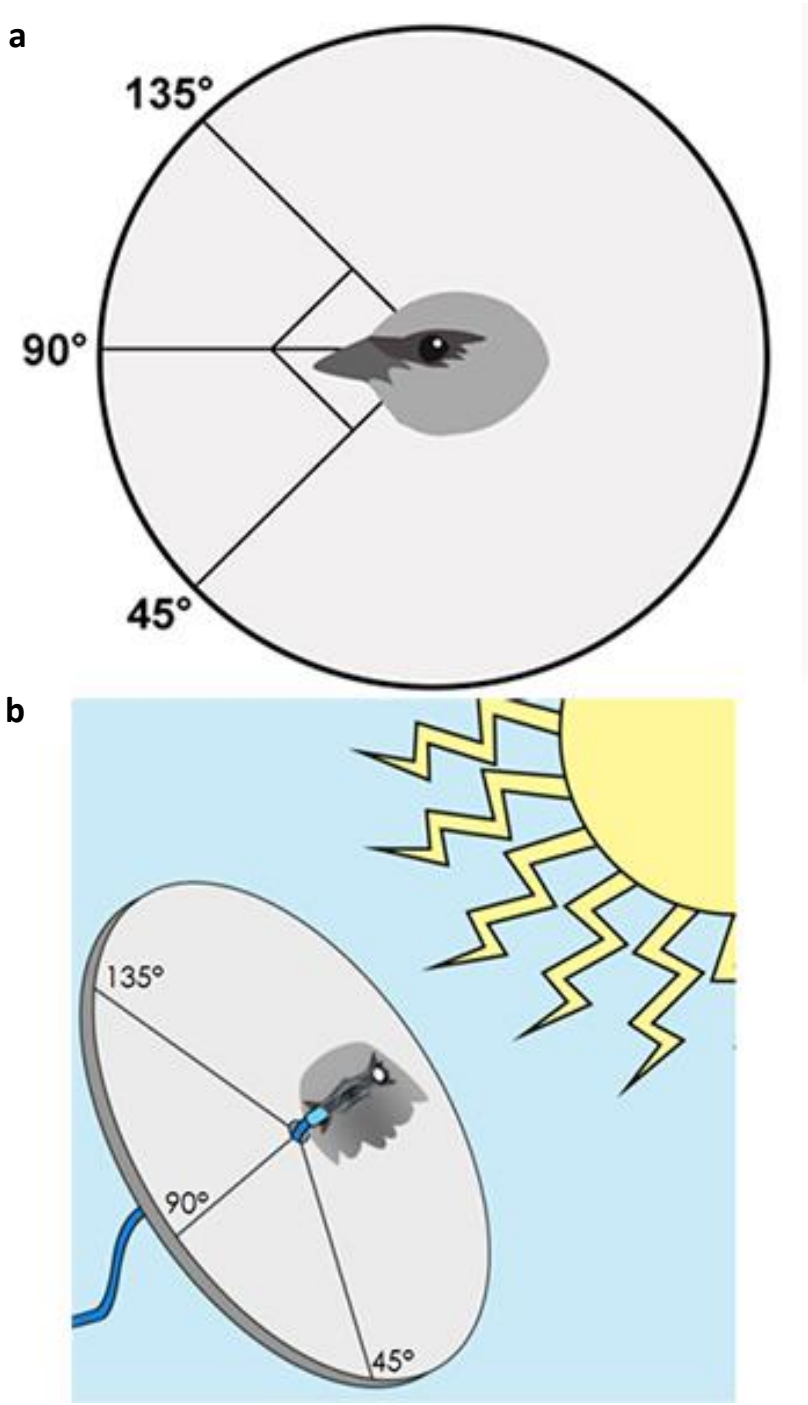


Figure 5. Two measurements (with the bird and a baseline reading without the bird) were taken at each tilt (135°, 90°, and 45°) (a) for each bird while maintaining the probe's perpendicular view of the sun (b).

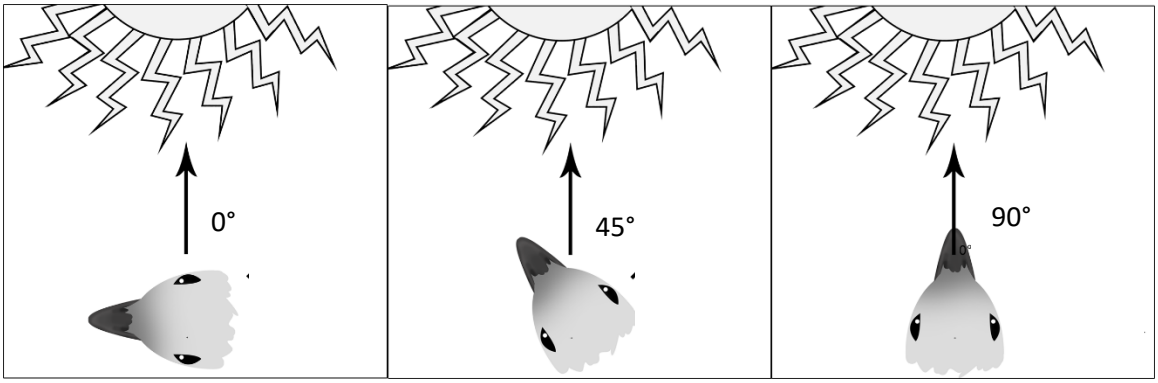


Figure 6. At 0° the eye faced directly into the sun, at 45° the eye was rotated 45° away from the sun, and at 90° the head was rotated so that the beak faced directly into the sun. All of the angles above were repeated with each specimen.

Statistical Methods

Statistical analyses were conducted using JMP v.14 (SAS Institute Inc., Cary, NC). Welch's t-tests, which assume normality but unequal variances and are robust to Type 1 errors, were used to test whether species with facial masks (this includes birds with all dark heads) had reduced irradiance compared to those without (Delacre et al. 2017). Simple linear regression analyses were used to examine the potential relationships of the following explanatory predictor variables on reduction in brightness: average head brightness, percent brightness of each quarter, patch index values, and bill index values (Mills et al. 1991). For this study, reduction in brightness was calculated from changes in irradiance between baseline irradiance measurements (without the specimen) and irradiance measurements with the specimen. Positive values of reduction in brightness imply lower irradiance was measured with specimens compared to

ambient levels, and negative values imply higher irradiance was found with specimens versus ambient levels. Regressions were performed on untransformed variables and assumptions were evaluated with graphical residual plots; significance in the regressions were based on the F-ratio test statistic (Mills et al. 1991). Relationships between predictors and responses were also analyzed with a non-parametric method, Spearman's Rho(ρ_{sp}), for which variables do not need to be normally distributed or have a linear relationship (Ducatez and Lefebvre 2014). Analyses were completed for each head angle. Correlations between multiple predictor variables were identified using a principal component analysis (PCA) which parses the correlation among the predictor variables with eigenvalues and eigenvectors (Sodhi et al. 1999; Robinson-Wolrath and Owens 2003). Significance for all analyses were based on α-values below 0.05 (Mills et al. 1991). I did not use a method such as the Bonferroni correction, when calculating significance values, in order to reduce the potential of Type 2 errors (the possible erroneous acceptance of a false null hypothesis; Armstrong 2014).

RESULTS

The data indicate that certain facial and bill morphologies have the potential to reduce glare at certain head angles. Birds with dark facial masks showed significant reductions in irradiance (though all measurements were above 0) when their eye location faced the sun (0° head rotation) and at all three angles: 45°, 90°, and 135° (Table 1, Appendix A). When the eye angled away from the sun at 45° and 90° there were no significant differences between masked birds and non-masked birds for reduction in brightness (Table 1). Standard error increased as reduction in brightness values increased (Table 1, Fig. 7).

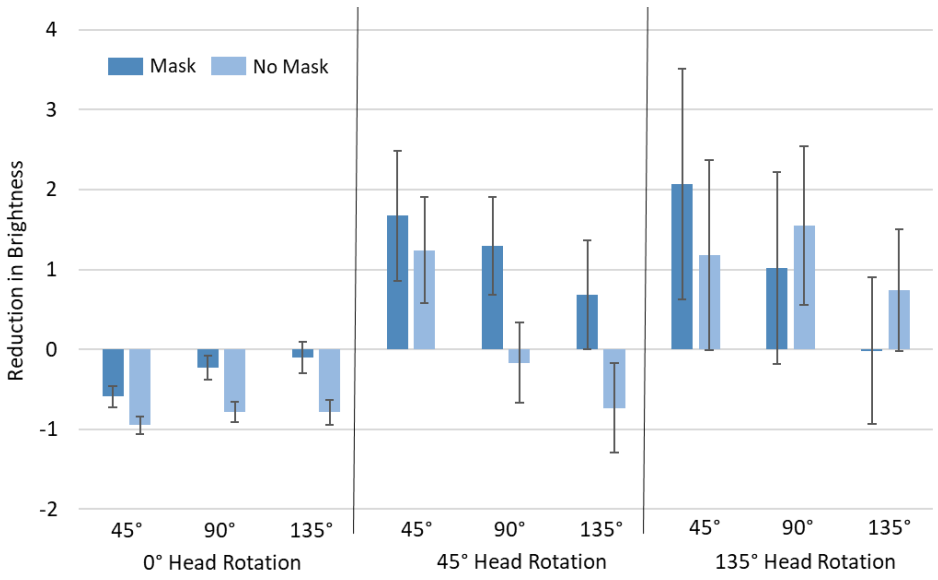


Figure 7. Reduction in brightness means, with error bars, for birds with dark facial masks and no facial masks.

Table 1. Welch's t-tests were used to examine the effects of dark facial masks on reduction in brightness by comparing birds with masks to those without; bold P-values and t-test statistics indicates significance.

		Mask	No Mask	Welch's t-Test	
Head Rotations	Head Tilts	Mean, Reduction in Brightness (% ± S.E.)	Mean, Reduction in Brightness (% ± S.E.)	*T*	*P*
0°	45°	-0.593 ± 0.133	-0.948 ± 0.110	**2.214**	**0.033**
	90°	-0.229 ± 0.155	-0.779 ± 0.128	**2.757**	**0.009**
	135°	-0.103 ± 0.192	-0.787 ± 0.158	**2.694**	**0.011**
45°	45°	1.670 ± 0.809	1.241 ± 0.668	0.452	0.654
	90°	1.294 ± 0.607	-0.169 ± 0.501	1.770	0.088
	135°	0.684 ± 0.682	-0.732 ± 0.563	1.579	0.125
90°	45°	2.064 ± 1.444	1.180 ± 1.192	0.425	0.675
	90°	1.018 ± 1.197	1.549 ± 0.988	0.331	0.742
	135°	-0.017 ± 0.919	0.742 ± 0.759	0.621	0.539

A higher proportion of dark facial patches, as indicated by the patch index, significantly minimized irradiance at certain angles. Birds with smaller patch index values exhibited significant reduction in brightness with head and eye locations facing the sun (0° head rotation) and a head tilt of 90° (Table 2), whereas birds with higher patch index values saw increased brightness entering the eye (Fig. 8a). With this eye angle (0° head rotation), no significant pattern of reduction in brightness was seen with the bill pointed up or down at the 45° or 135° head tilts (Table 2). When the eye was turned 45° from the sun (45° head rotation) all head tilts (45°, 90°, and 135°) exhibited significant reduction in brightness for birds with smaller patch index values (Table 2, Fig. 8b–d). No correlation was found with the patch index and reduction in brightness when the head was rotated away from the sun at 90° for any head tilt (Table 2).

Table 2. Tests were performed with simple linear regressions and spearman's rho on the patch index; P-values set in bold indicate statistical significance.

Head Rotation		Head Tilt								
		45°			90°			135°		
	Error DF	F-ratio	P-value	(ρ_{sp})	F-ratio	P-value	(ρ_{sp})	F-ratio	P-value	(ρ_{sp})
0°	35	2.99	0.092	-0.327	**5.57**	**0.024**	**-0.433**	1.58	0.216	-0.269
45°	35	**7.96**	**0.007**	**-0.497**	**7.76**	**0.008**	**-0.471**	**13.04**	**0.000**	**-0.513**
90°	35	1.85	0.182	-0.243	2.50	0.122	-0.139	0.49	0.486	0.011

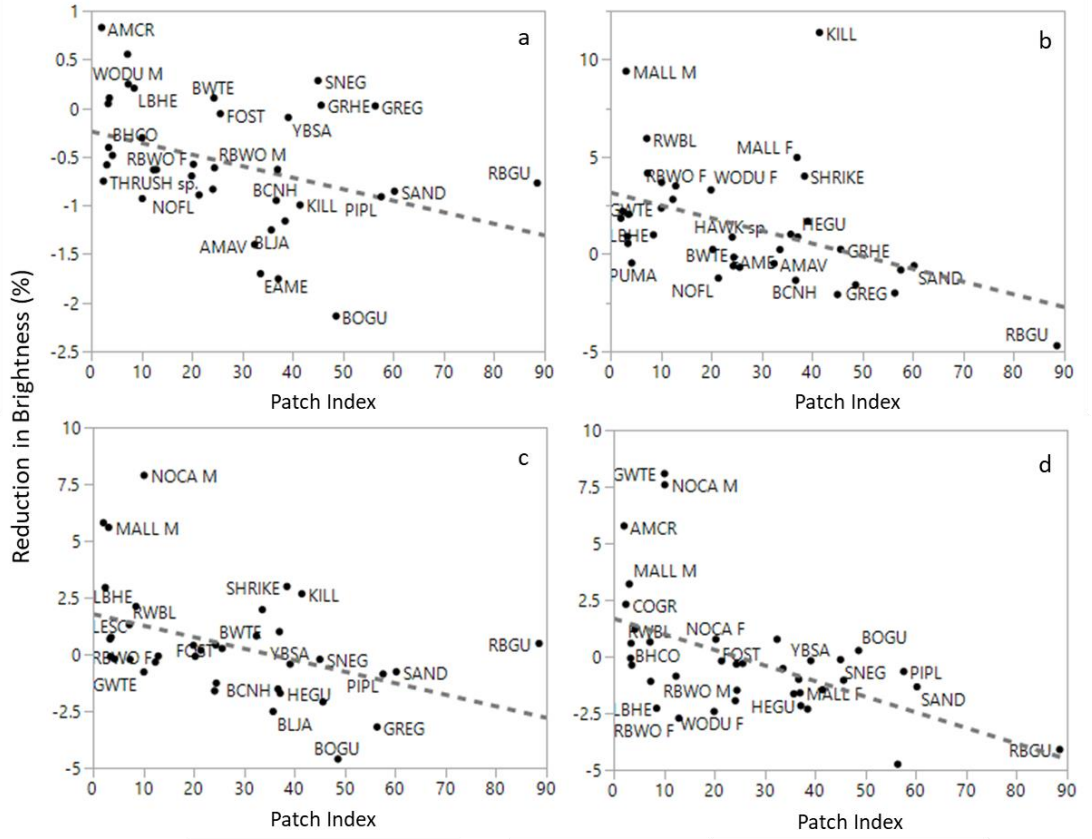


Figure 8. 0° Head Rotation (a). **45° Head Rotation** 45°(b), 90°(c), and 135°(d). The Patch Index formula is Patch x brightness × Patch x Proportion of area = Patch Index. Changes in absolute irradiance (µmol m^{-2} s^{-1}) between ambient and specimen have been converted to a percentage to represent reduction in brightness. Positive values of reduction of brightness imply lower irradiance was measured with birds compared to ambient levels, and negative values of reduction in brightness imply higher irradiance was found with the bird vs ambient levels. See Appendix A for bird species alpha codes.

Birds with overall darker heads, as measured by decreased average head brightness *(*average taken from all four quarters), demonstrated a significant decrease in brightness when their eye was directly facing the sun (0° angle) and angled 45° away from the sun (Table 3). Brightness was significantly reduced at the 0° head rotation when the head was tilted down at 45° and at 90° (Table 3, Figure 9a,b). However, reduction in brightness did not quite reach significance at the at this head rotation when the head was tilted up at 135°. At the 45° head rotation brightness decreased at all tested angles of tilt (Table 3, Fig. 9c–e). No correlation was found when a bird's beak directly faced the sun (90° head rotation; Table 3).

Table 3. Tests were performed with simple linear regressions and Spearman's rho on average head brightness; P-values set in bold indicate statistical significance.

Head Rotation		Head Tilt								
		45°			90°			135°		
	Error DF	F-ratio	P-value	(ρ_{sp})	F-ratio	P-value	(ρ_{sp})	F-ratio	P-value	(ρ_{sp})
0°	35	**5.4**	**0.026**	**-0.370**	**4.42**	**0.042**	**-0.442**	3.75	0.061	**-0.326**
45°	35	**9.2**	**0.004**	**-0.519**	**6.94**	**0.012**	**-0.359**	**7.72**	**0.008**	**-0.467**
90°	35	0.37	0.544	-0.298	1.77	0.191	-0.095	0.01	0.913	0.047

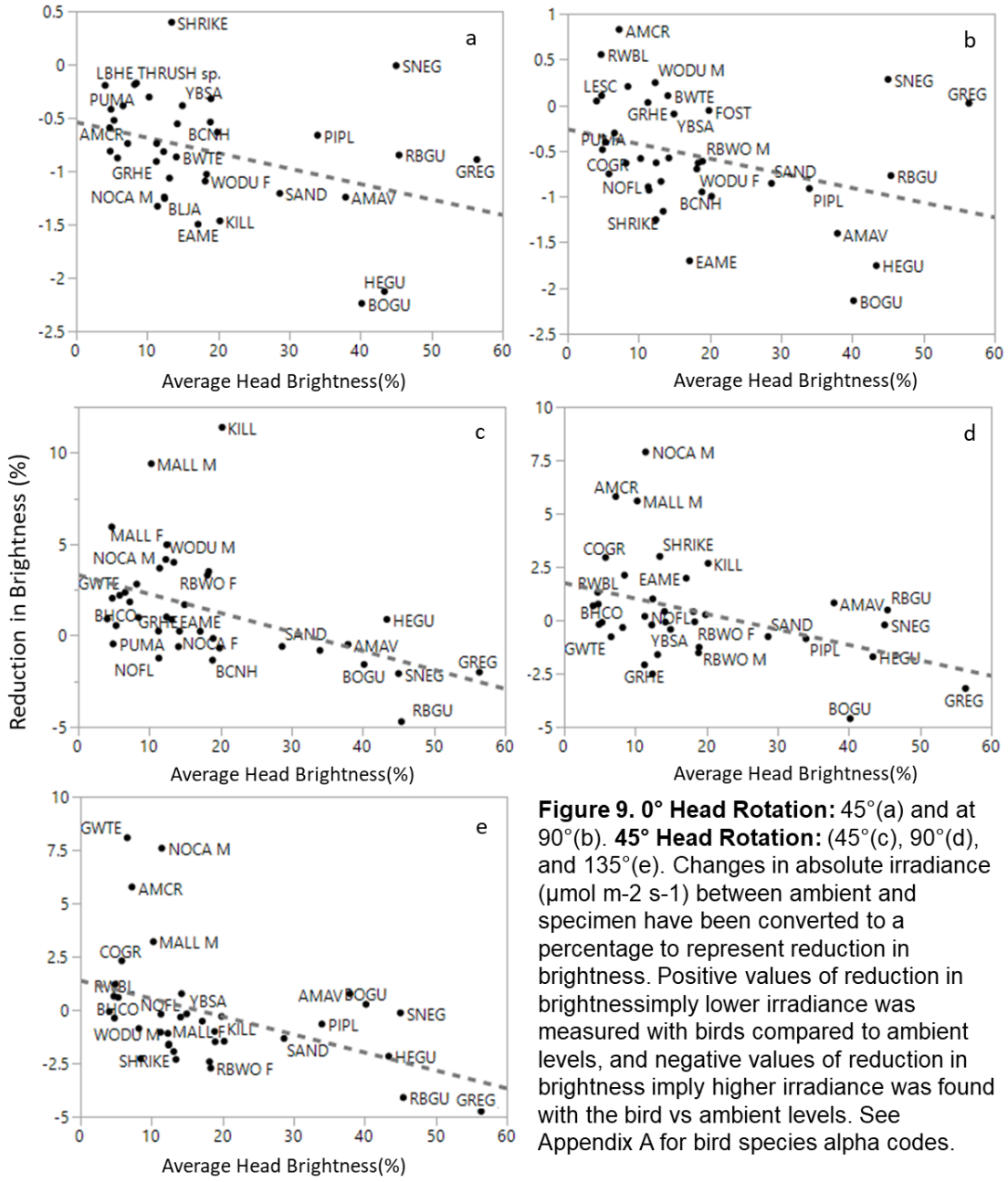


Figure 9. 0° Head Rotation: 45°(a) and at 90°(b). **45° Head Rotation:** (45°(c), 90°(d), and 135°(e). Changes in absolute irradiance (µmol m-2 s-1) between ambient and specimen have been converted to a percentage to represent reduction in brightness. Positive values of reduction in brightnessimply lower irradiance was measured with birds compared to ambient levels, and negative values of reduction in brightness imply higher irradiance was found with the bird vs ambient levels. See Appendix A for bird species alpha codes.

Average head brightness showed varying relationships with light entering the eye across the different facial quarters (Table 4). When the eye faced directly into the sun (0° head rotation) birds with darker plumage in quarters 1 and 3 (plumage dorsal and anterior the eye, and plumage posterior and ventral to the

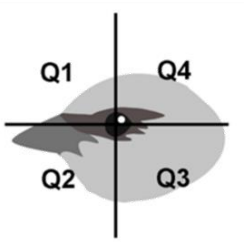
eye) showed significant reduction in brightness with the head was tilted at 90° (Table 4). With this same eye direction, birds with darker plumage in quarters 1, 3, and 4 (all plumage except that which is anterior and ventral to the eye) had significantly more reduction in brightness when their head was tilted down at 45° (Table 4). And birds with their heads tilted up at 135° saw significant reduction in brightness from darker plumage in quarter 4 (plumage posterior and dorsal to the eye) (Table 4). No significant results were found for quarter 2 at any foraging angle (Table 4).

When the eye was angled 45° from the sun (45° head rotation) dark plumage in all quarters reduced brightness at most head tilts, but only those supported by a significant *P*-value and Spearman's Rho are reported in text. When the bird's head was tilted down at 45° dark plumage in all quarters (1, 2, 3, and 4) significantly reduced brightness (Table 4). Birds with their heads tilted at 90° showed significant reduction in brightness from dark plumage in quarters 3 and 4 (plumage posterior to the eye; Table 4). Birds with dark plumage in quarters 1, 3, and 4 (all plumage except that anterior and ventral to the eye) had significant reduction in brightness with the head tilted up at 135° (Table 4). No correlation was found between reduction in brightness and dark plumage in any quarter when a bird's head was rotated at the 90°head rotation when the bill faced directly into the sun (Table 4).

Additionally, it can be expected that some quadrats are correlated, but they are analyzed separately to show if location of dark plumage around the eye

shows any relationship to reduction in brightness. Any correlation between quadrats will vary based on the individual species plumage, however the goal of this analysis is to examine overall patterns of dark plumage across several species.

Table 4. Tests were performed with simple linear regressions and Spearman's rho on brightness of quarters 1-4; bold text indicates significance. Figure 2 is repeated for reference.



		0° Head Rotation								
Facial Plumage Quarter	Error DF	45° Head Tilt			90° Head Tilt			135° Head Tilt		
		F-ratio	P-value	(ρ_{sp})	F-ratio	P-value	(ρ_{sp})	F-ratio	P-value	(ρ_{sp})
Q1	35	**7.23**	**0.011**	**-0.325**	**5.15**	**0.029**	**-0.388**	4.06	0.051	-0.264
Q2	35	2.48	0.124	-0.317	1.55	0.221	-0.338	1.91	0.175	-0.271
Q3	35	**4.30**	**0.045**	**-0.349**	**6.09**	**0.018**	**-0.441**	2.64	0.113	-0.280
Q4	35	**5.13**	**0.029**	**-0.421**	3.24	0.080	-0.436	**4.74**	**0.036**	**-0.485**
		45° Head Rotation								
Facial Plumage Quarter	Error DF	45° Head Tilt			90° Head Tilt			135° Head Tilt		
		F-ratio	P-value	(ρ_{sp})	F-ratio	P-value	(ρ_{sp})	F-ratio	P-value	(ρ_{sp})
Q1	35	**8.19**	**0.007**	**-0.486**	**6.82**	**0.013**	-0.295	**4.28**	**0.045**	**-0.342**
Q2	35	**9.36**	**0.004**	**-0.562**	**4.31**	**0.045**	-0.275	**6.29**	**0.017**	-0.289
Q3	35	**8.42**	**0.006**	**-0.509**	**5.69**	**0.022**	**-0.366**	**9.58**	**0.003**	**-0.505**
Q4	35	**6.22**	**0.017**	**-0.366**	**7.26**	**0.010**	**-0.434**	**7.65**	**0.009**	**-0.489**
		90° Head Rotation								
Facial Plumage Quarter	Error DF	45° Head Tilt			90° Head Tilt			135° Head Tilt		
		F-ratio	P-value	(ρ_{sp})	F-ratio	P-value	(ρ_{sp})	F-ratio	P-value	(ρ_{sp})

Table 4, continued.

Q1	35	0.29	0.590	-0.296	1.63	0.210	-0.034	0.001	0.974	0.037
Q2	35	0.09	0.756	-0.211	1.49	0.228	-0.126	0.23	0.635	0.024
Q3	35	1.20	0.280	-0.291	2.15	0.151	-0.121	0.10	0.748	0.049
Q4	35	0.10	0.752	-0.207	1.07	0.307	-0.063	0.08	0.776	0.064

Bill coloration showed no correlation with reduction in brightness in this study (Table 5). Only 28 specimens were used for this analysis; some species were excluded based on bill size (too small for the probe to accurately measure reflectance) or bill discoloration (Appendix A).

Table 5. Tests were performed with simple linear regressions and Spearman's rho on bill brightness; bold text indicates significance (none denoted in table).

Head Rotation		Head Tilt								
		45°			90°			135°		
	Error DF	F-ratio	P-value	(ρ_{sp})	F-ratio	P-value	(ρ_{sp})	F-ratio	P-value	(ρ_{sp})
0°	35	0.005	0.943	-0.152	0.024	0.877	-0.074	0.143	0.707	-0.171
45°	35	0.005	0.940	-0.193	3.11	0.089	0.350	0.39	0.539	0.145
90°	35	0.44	0.512	-0.212	1.47	0.235	-0.208	1.97	0.171	-0.137

Principal Components Analysis of Reduction in Brightness Predictor Variables

Although simple linear regressions were run to examine the relationships between reduction in brightness and the predictor variables it is understood these predictor variables are not independent. Three categories of reduction in brightness predictor variables (average head brightness, percent brightness of each quarter, and patch index values) were highly correlated with each other, but each showed no correlation with bill index (Table 6, Fig. 10). The lack of

correlation between these variables is also visible in the grouping of the predictor variables into the principal components (Table 7). Principal component 1 (PC 1) is equally influenced by the patch index, all quarters, and average head brightness and accounts for 70.3% of the variance (Table 7, Fig. 10). Principal component 2 (PC2) is almost completely dominated by the bill index and explains 16.9% of the variance (Table 7, Fig. 10).

Table 6. Correlation matrix of reduction in brightness predictor variables. Correlations range from 1 to -1, with numbers closer to 1 or -1 indicating a strong correlation and numbers closer to 0 indicating little to no correlation. Bold numbers indicate significance. Portions of the head (Q1-Q4) are indicated as in Fig. 2.

	Bill Index	Patch Index	Q1 brightness (%)	Q2 brightness (%)	Q3 brightness (%)	Q4 brightness (%)
Bill Index	**1**	-0.075	0.048	0.031	-0.049	-0.065
Patch Index		**1**	**0.675**	**0.791**	**0.800**	**0.670**
Q1 Brightness (%)			**1**	**0.848**	**0.862**	**0.887**
Q2 Brightness (%)				**1**	**0.854**	**0.791**
Q3 Brightness (%)					**1**	**0.844**
Q4 Brightness (%)						**1**

Table 7. Eigenvalues and eigenvectors comparing reduction in brightness predictor variables. Eigenvalues express the amount of variance explained by each principal component. Eigenvectors show the weight of each variable on each principal component. Bold text indicates which eigenvectors had a major influence on each principal component.

Eigenvalues			
PC#	Eigenvalue	Variance (%)	Cumulative Variance (%)
1	4.216	70.281	70.281
2	1.016	16.935	87.216

Eigenvectors of the Principal Components		
Variables	PC1	PC2
Bill index	-0.014	**0.989**
Patch Index	**0.415**	-0.075
Q1 brightness %	**0.454**	0.093
Q2 brightness %	**0.454**	0.067
Q3 brightness %	**0.463**	-0.025
Q4 brightness %	**0.445**	-0.034

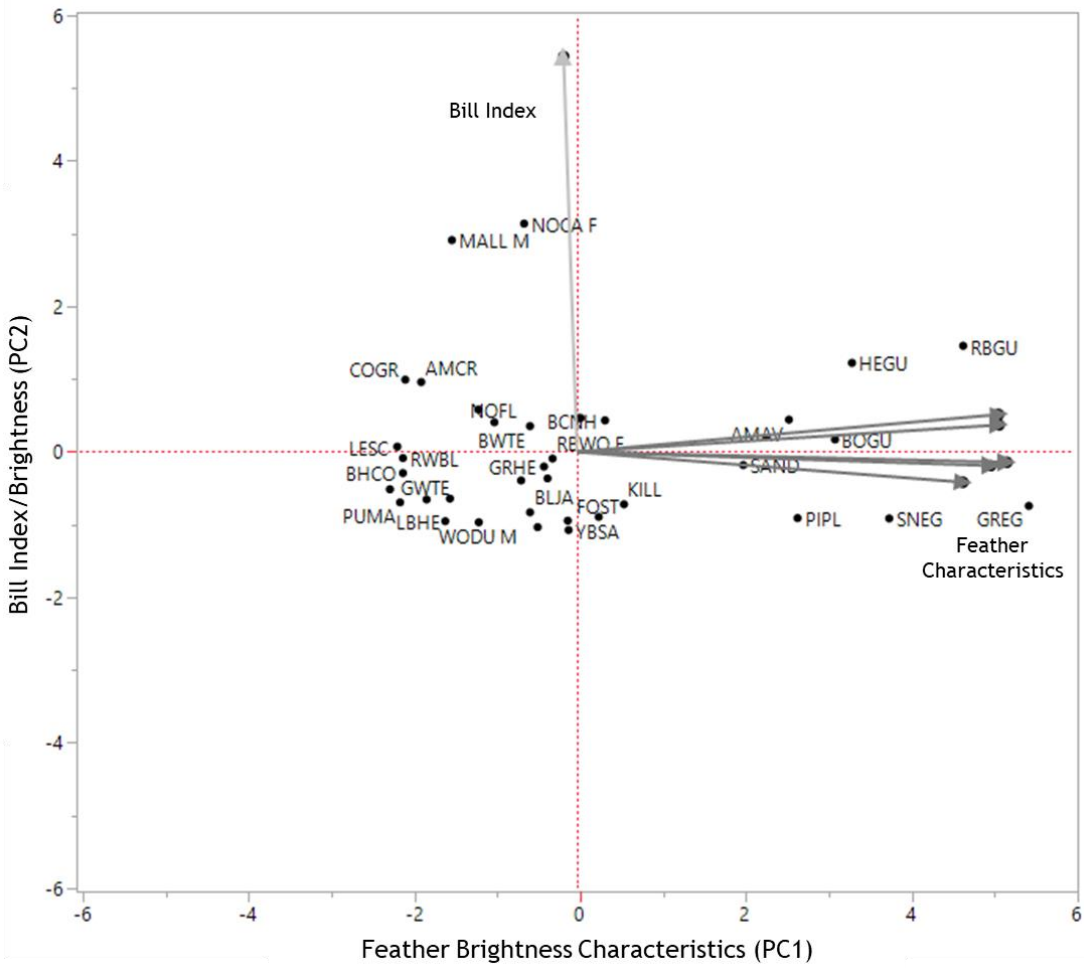


Figure 10. PCA plot. Axes show ranges of principal component scores associated with the first two eigenvectors. A score indicates how a particular observation weighs on a particular eigenvector. Principal component score is calculated by multiplying the observations' predictor values by the principal component eigenvectors. See Appendix A for bird species alpha codes.

DISCUSSION

This study demonstrates several conditions in which dark facial patterns significantly reduce the amount of light entering the eye. Dark facial masks significantly decreased glare for birds when the eye faced directly into the sun (0° head rotation), but not at the other head rotations. Birds with larger dark facial patches and with overall darker heads saw some reduction in brightness when the eye was positioned towards the sun (0° head rotation) and when the eye was angled 45° away from the sun (45°head rotation). The varying significant results between the facial masks and other predictor variables could be related to how these traits are qualified. Average head brightness, quarter brightness, and the patch index are all gradients of light to dark plumage, whereas the presence or absence of facial masks is dichotomous (large and small dark facial masks are rated the same).

Having darker plumage diminished the amount of light entering the eye consistently in all analyses that focused on individual quarters when the eye was rotated away from the sun at 45°. This was also true for quarters 1, 3 and 4 at most head tilts when the eye directly faced the sun (0° head rotation). When the head was tilted up at 135°, however, only dark plumage in quarter 4 (plumage

posterior and dorsal to the eye) lessened glare at the 0° head rotation. There was no indication of reduction in brightness at any head tilt with the head rotated away from the sun at 90 degrees. It appears that dark plumage on the top of the head and dark plumage posterior to the eye might have the greatest potential to aid in reducing glare for some species.

The presence of dark facial masks might aid in foraging for food in bright environments with the head held at certain angles if there is a reduction or altering in the intensity of light entering the eye (Yosef et al. 2012). Dark plumage might be especially advantageous when the eye faces the sun or angles slightly away from it (head rotations of 0° and 45°) and the head is held horizonal (90° head tilt) or down at 45° for bird species that glean food sources from the ground or forage from branches (Remsen and Robinson 1990; Carr and Lima 2014).

The American Crow specimen in this study experienced reduction in brightness at most angles of the 0° and 45° head rotations. American Crows have shown a preference for foraging in sunny habitats over shady habitats during winter and no preference during the summer (when there is no thermoregulatory advantage to either habitat; Kilpatrick 2003). It is possible their dark plumage is advantageous by allowing the crow to both warm up faster in the winter and to see more effectively in these sunny habitats with high glare potential.

These plumage patterns might also be advantageous to species such as raptors and shrikes that dive to catch their prey in brightly lit habitats where the combined benefit of reduction in brightness along with their countershading could increase hunting success (Smithwick et al. 2017). The Loggerhead Shrike (*Lanius ludovicianus*) specimen used in this study saw some minor reduction in brightness with its eye directly facing the sun (0° angle) with the head tilted down at 45° and up at 135° (though at 90° the shrike saw increased light entering the eye). With the head rotated at 45°, there was greater reduction in brightness for the shrike at head tilts of 45° and 90°. Reduction in brightness at these angles for shrikes might aid in tracking and striking terrestrial prey. Future research could explore if this trend applies to other shrike species, especially as previous research has shown that masked shrikes are more likely to strike at prey when facing towards the sun if their masks are unaltered (Yosef et al. 2012).

Dark plumage might also aid in other aspects of a bird's life. A bird tilting its head horizontally at 90° and up to 135° might find reduction in brightness from dark plumage helpful when flying. Some species, like certain terns, have long distance migrations and maintain large dark facial patches year-round (Voelker 1996). The Forster's Tern (*Sterna forsteri*) specimen used in this study experienced some reduction in brightness at these head tilts when its eye directly faced the sun (0° angle) or angled slightly away from the sun (45° angle). Minimizing glare at these angles might allow the bird to better navigate, react faster to danger, or forage more efficiently in bright aquatic environments.

Increased light entering the eyes in sunny areas can make it difficult for birds to detect predators (Fernández-Juricic et al. 2012). Prior research has found that Brown-headed Cowbirds (*Molothrus ater*) reacted to predators more slowly when foraging in sunny areas (Fernández-Juricic et al. 2012). The advantage of reduction in brightness for some birds with dark colored facial markings may aid in faster reaction times than birds without the dark plumage. The Brown-headed Cowbird specimen in this study experienced a small amount of reduction in brightness when its head was tilted down at 45° and up at 135°, it is possible their dark plumage may confer them some advantage when foraging in sunny areas and scanning for predators.

In contrast to the patterns above, some shorebirds and wading birds, such as the Great Egret *(Ardea alba),* have light colored plumage and frequently forage in brighter areas (Brown 2017). The Great Egret did not exhibit reduction in brightness in this study except when its beak directly faced the sun (90° head rotation), when shading from head orientation may play a greater role in reduction in brightness than plumage color. The role of bill brightness in reducing light entering the eyes for this species is unclear on account of specimen bill discoloration. Additionally, it has been hypothesized that an interior eye structure, the pecten, which can be heavily pigmented, might reduce the incident light that interferes with vision in this type of bird (van den Hout and Martin 2011; Brown 2017).

Sex- and age-based facial plumage dimorphism might allow intraspecific niche partitioning (Rohwer et al. 1983). For example, Rohwer et al. (1983) speculated that male American Redstarts (*Setophaga ruticilla*) gained an advantage over females and young adults through darker plumage that enables them to forage in sunnier areas. Earlier research with warblers noted facial plumage sexual dichromatism, but did not address this factor when collecting field data (Burtt 1986). Overall plumage coloration of barn owls affects hunting success (San-Jose et al. 2019). On nights of the full moon, owls with whiter plumage can cause voles to freeze for longer periods of time prior to the owl's attack, thereby increasing its chance of catching the prey (San-Jose et al. 2019). Additional studies of intraspecific plumage morphology and variation in foraging success or foraging strategies between the sexes and age groups are warranted.

Bill color did not reduce glare in the specimens used in this study and varied from the other reduction in brightness predictor variables according to the PCA (Tables 6-7, Fig. 10). Previous field studies suggest that darker bill color plays a prominent role in reduction in brightness in some flycatcher and warbler species while foraging (Burtt 1984, 1986). Differences in results between this and previous studies might be attributable to methodological limitations in this study. First, bills of museum specimens may not be representative of bills of live birds. While feathers are composed entirely of dead material and do not differ between living birds and well-preserved museum specimens, bill color is influenced by living tissues and bill color of certain species may fade after death

(Armenta et al.2008; Graves 2009). Additionally, the equipment utilized in this study was unable to measure bills below a certain size threshold. It is possible that having accurate bill reflectance for all of the species in this study could have yielded different results because several of the smaller species in this study had darker bills. Future research should use of a finer probe, which might make it possible to gain readings from these smaller species.

Another limitation of this study, attributable to the relatively large size of the probe used to record irradiance, is that it prevented examination of species with small skulls and eyes. Several species that were the focus of previous research (warblers and flycatchers; Burtt 1984, 1986), have skulls and eyes that are too small for the cosine corrector probe used in this study. Additionally, larger species with eye openings far greater in size than the cosine corrector cannot give accurate readings as the feathers around the eye will not be immediately adjacent to the probe.

Although the amount of reduction in brightness by dark plumage demonstrated in this study is small, this could provide a selective advantage in the context of foraging or predator detection. Other factors not considered in this study may accentuate reduction in brightness. Eyebrow ridges and protruding feathers may block sunlight from entering the eye in some species and further increased reduction in brightness (Martin and Katzir 2000) while protecting the eye from dust or other irritants (Jones et al. 2007). Future research could consider these factors, and address the importance and placement of dark

patches and bills with different foraging modes guilds. Some foraging guilds may find dark plumage in certain locations more useful than other guilds might. Under the correct circumstances, such as birds foraging in open sunlit areas, dark facial features might represent a subtle, but underappreciated adaptive, morphology.

CHAPTER II

FEATHER MICROSTRUCTURAL FEATURES AFFECTING PLUMAGE BRIGHTNESS

INTRODUCTION

Feathers are extremely versatile structures, with adaptive roles that include waterproofing, insulation, flight surfaces, and ornaments used in communication (Burtt 1986; Ortolani 1999; Théry 2006; Maia et al. 2011; Yosef et al. 2012). Feather coloration is often a key component for feather functionality. Feathers prone to abrasion or needing extra strength typically have greater deposits of melanin (Averill 1923, Burtt 1979). Dark feathers also provide thermal benefits in colder environments (Margalida et al. 2008; Rogalla et al. 2019). The color of melanins can vary from red to black and they are frequently used as a base color in dark plumage patches that serve as social signals (McGraw et al. 2005; McGraw 2008). Additionally, dark head plumage has been shown to function in glare reduction (Burtt 1986; Yosef et al. 2012; Chapter 1). Whereas previous research has demonstrated this adaptive role of feather coloration to glare reduction (Burtt 1986; Yosef et al. 2012), no studies have explored the role of feather microstructure in glare reduction.

Feather structural characteristics have been shown to drive feather coloration and brightness (Galván 2011; Igic et al. 2018; McCoy et al. 2018). Feather coloration is largely produced by three components: pigments, structural colors, or the interplay of structural colors with pigments (Doucet et al. 2006).

Each of these three components working with the additional factors of viewing angle and lighting create the array of plumage colors that are visible to the human eye (Brink and van der Berg 2004; Doucet et al. 2006; Meadows et al. 2012; Van Wijk et al. 2016). Microscale arrangements of feather structural characteristics and pigments help produce a wide diversity of feather color and range of feather brightness visible across avian species (Brink and van der Berg 2004; Doucet et al. 2006; Maia et al. 2011; Meadows et al. 2012; Van Wijk et al. 2016).

The essential components of a feather are the central shaft, or rachis, which has lateral branching barbs (Fig. 1). In turn, these barbs have proximal (directed to the feather's base) and distal (directed away from the feather's base) branching barbules that branch off the barb's central shaft or ramus (Fig. 1; Prum 1999; Dove and Koch 2011; Harvey et al. 2013). The barbules of neighboring barbs overlap and are held together by hooklets on the distal barbules, which interlock with proximal barbules of another barb, in feathers this forms an integrated vane or pennaceous feather region (Fig. 1; Prum 1999; Dove and Koch 2011; Harvey et al. 2013). A barb's ramus has two main internal layers, an inner pith and outer cortex (Fig. 1; Galván 2011; Dove and Koch 2011; Harvey et al. 2013). Typically, the cortex appears more solid and the pith is filled with a spongy matrix of keratin and air pockets (Igic et al. 2018).

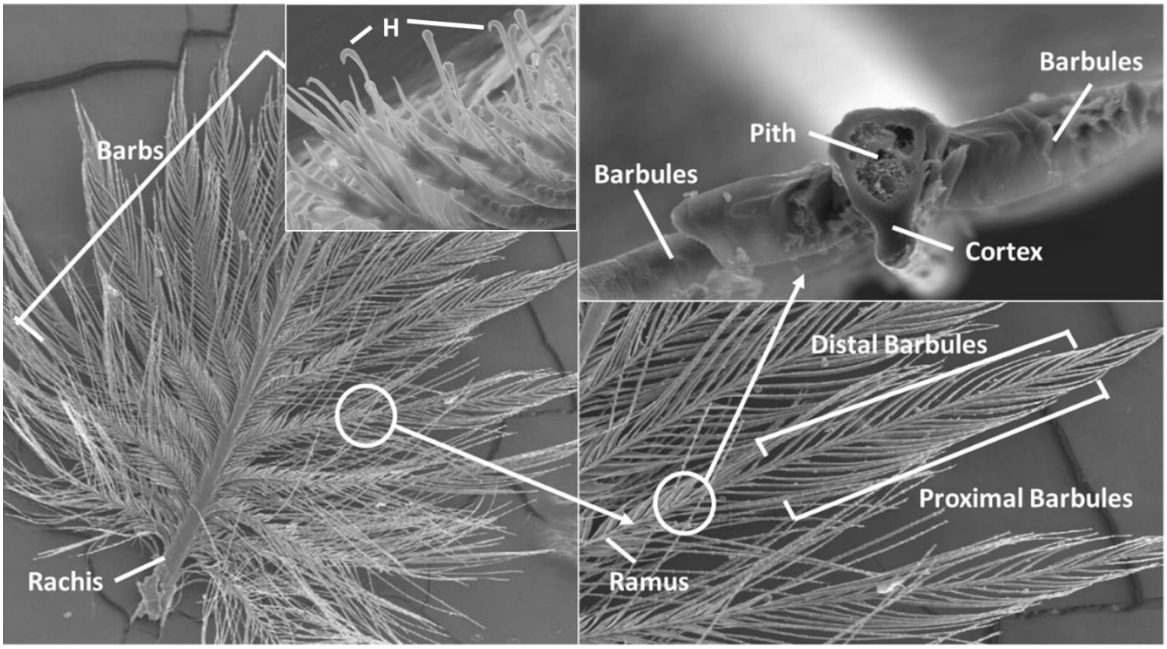


Figure 1. A pennaceous feather with central shaft, or rachis, hooklets (H), and barbs branching on either side of the rachis. For each barb its central shaft, or ramus, has both distal and proximal branching barbules. Within the barb ramus are two layers, the pith and cortex.

Some of the showiest feather colors are created not solely through pigments, but through specific compositions of external and internal microstructural feather elements (Greenewalt et al. 1960; Doucet 2002; Doucet et al. 2006). The iridescence of hummingbird feathers is made by combinations of hollow melanosomes, an array of keratin and melanin granules (Greenewalt et al. 1960; Meadows et al. 2012; Van Wijk et al. 2016; Eliason et al. 2020). Dark feathers characteristics in particular might be driven by both pigments and characteristics of the feather microstructure (Lee et al. 2009, 2010; Galván 2011; D'Alba et al. 2014). The primary pigments used by birds with dark feathers are melanins (McGraw 2008). Production of melanistic feathers in birds is influenced

by a number of different processes, including the use of different metals found in the animal's diet or hormones that can be impacted by social interactions (McGraw 2008). Feather microstructure, including the positioning of melanin granules within barbs and barbules, also impacts feather coloration (Lee et al. 2009, 2010; Galván 2011; D'Alba et al. 2014). For example, the dark colorations of Great Tits (*Parus major*) and Black-capped Chickadees (*Poecile atricapillus*) are chiefly produced by the pigment melanin and structural traits (Lee et al. 2009; Galván 2011; D'Alba et al. 2014).

Differences in feather microstructure among species can create varying shades of black plumage. For example, the ultra-dark birds of paradise (family Paradisaeidae) can absorb more light than the American Crow (*Corvus brachyrhynchos*). The black feathers of several birds of paradise species such as the Paradise Riflebird (*Ptiloris paradiseus*) and Stephanie's Astrapia (*Astrapia stephaniae)*, can absorb over 99% of light because of their unique microstructures, while the American Crow can absorb around 90-93% (McCoy et al. 2018; Chapter 1). The microstructure of the black feathers can function as an indicator of bird health and quality (D'Alba et al. 2014).

The brightness of white feathers is also attained through microstructural features that can vary across species (Igic et al. 2018; Stuart-Fox et al. 2018). Chief among the microstructural characteristics that impact plumage brightness are barb and barbule density, and the internal structure of the barb ramus (Dyck 1979; Igic et al. 2018). The disorderly arrangement of these internal structures

causes incoherent scattering of light wavelengths, which gives rise to the white plumage coloration (Dyck 1979; Igic et al. 2018; Stuart-Fox et al. 2018). Larger birds are capable of producing larger feathers which allowed them to have more complex layers within barb rami, and subsequently brighter white feathers (Igic et al. 2018). Increased barbule density is also correlated with greater white brightness levels (Igic et al. 2018; Stuart-Fox et al. 2018).

Gray feathers can be achieved through a variety of pigments and structural effects. The gray colored feathers of the Dark-eyed Junco (*Junco hyemalis*) are the result of small amounts of melanin. Some corvid species produce gray or blue-gray feathers through eumelanin and barb coloration rather than barbule coloration (Lee et al. 2016). The gray morph of the Tawny Owl (*Strix aluco*) has greater plumulaceous barbule density than the brown morph (Koskenpato et al. 2016; de Zwaan et al. 2017).

Feather structural characteristics may play a proximate role in the glare reduction phenomena documented in some birds. Darker plumage has been shown to decrease glare more so than light plumage in both laboratory and field studies. Masked Shrikes angle away from the sun when diving at prey if their standard dark facial masks have been altered (Yosef et al. 2012). The Loggerhead Shrike (*Lanius ludovicianus*), which also has a black facial mask and uses similar hunting strategies, demonstrates reduction in brightness potential at several head angles in laboratory analyses (Chapter 1). On the other hand, birds

with lighter colored plumage surrounding the eye have increased light gathering potential at several head angles (Chapter 1).

Quantification of Feather Microstructure and Feather Coloration

The avian visual system enables birds to see colors beyond what is visible to humans (Doucet 2002; Eaton and Lanyon 2003; Doucet et al. 2006). On average, humans can see wavelengths of light between 400-700 nm, but several bird species are able to see wavelengths down to 300 nm, which encompasses a portion of the ultraviolet spectrum (Doucet 2002; Eaton and Lanyon 2003; Doucet et al. 2006). Because these wavelengths are invisible to the human eye many researchers are turning to spectroscopy to study plumage coloration and avian visual systems (Doucet 2002; Doucet et al. 2006; Lee et al. 2009). Use of a spectroscopy equipment allows scientists to get an accurate and unbiased view of the color properties of a bird's plumage and bill.

Several researchers have found that barbule shape, barbule density, and ramus characteristics impact how light bounces off a feather (Brink and van der Berg 2004; Doucet et al. 2006; Galván 2011; D'Alba et al. 2014; McCoy et al. 2018). For example, several different species of birds of paradise like the Superb Bird-of-Paradise (*Lophorina superba*), Twelve-wired Bird-of-paradise (*Seleucidis melanoleucus*) and others had significantly darker feathers than other closely related species (McCoy et al. 2018). Feathers taken from museum specimens of birds of paradise and species with typical black plumage were

examined with scanning electron microscopy (McCoy et al. 2018). The barbule structures of the birds of paradise were drastically different than other black birds, in that they curve up and have mountains and valleys created by their serrated shape (McCoy et al. 2018). Utilizing a nano-CT scanner and 3D models of the feathers, researchers found that birds of paradise feathers have more structural absorbance and thus appear darker than the average black feather (McCoy et al. 2018).

In addition to their shape, other features of barbules can influence feather color (Galván 2011; Igic et al. 2018; Lee et al. 2009, 2010). Barbule density plays an important role in feather brightness (Lee et al. 2009, 2010; Galván 2011, D'Alba et al. 2014; McCoy et al. 2018; Laczi et al. 2019). Dark plumage in black color patches of Great Tits was correlated with greater barbule density (Galván 2011; Laczi et al. 2019). Darker feathers were also associated with greater barbule density for Zebra Finches (*Taeniopygia guttata*) and Black-capped Chickadees (D'Alba et al. 2014). In contrast, other studies have found associations between bright white plumage and increased barbule density (Igic et al. 2018; Stuart-Fox et al. 2018). When determining barbule density, it is key to differentiate between barbules on the proximal (directed towards the feather's base) and distal (directed away from the feather's base) sides of the barb as differences between the two sides can create unique visual effects (Galván et al. 2009; Shawkey et al. 2011). For example, in both the Bearded Vulture (*Gypaetus barbatus*) and Anhinga (*Anhinga anhinga*) distinctions between the proximal and

distal barbules produce a pearlescent silvery color (Galván et al. 2009; Shawkey et al. 2011). Distal barbules of the Anhinga's silvery feathers are much longer than their proximal barbules, and possess different coloration and internal layers (Shawkey et al. 2011). The Bearded Vulture has similar lengthened distal barbules, with a distinctive twisted morphology (Galván et al. 2009). Additionally, distal barbules are typically darker than proximal barbules for certain species (Lloyd-Jones 1915; Prum and Williamson 2002; Field et al. 2013). Considering both proximal and distal barbules as a single unit could conceal patterns of color and brightness in feathers.

Characteristics of a barb's ramus are also integral to producing feather colors (Brink and van der Berg 2004; Doucet et al. 2006; Galván 2011; Igic et al. 2018). In general terms, the ramus is made of two parts, the pith and cortex, and similar to plant anatomy the pith is incased inside the cortex (Galván 2011). Different features of the ramus can produce varying affects, such as the specific arrangement of melanin granules in the pith or a thick layer of keratin surrounding the cortex can both produce iridescence (Brink and van der Berg 2004; Doucet et al. 2006; Lee et al. 2009; D'Alba et al. 2014). For the feathers of the black breast patch of Great Tits, larger cortexes, smaller piths, and greater pith:cortex size ratios were all associated with darker plumage (Galván 2011). The same pattern can also produce brighter plumages. A comprehensive interspecies analysis of 61 avian species with white plumage found an overall

trend that greater reflectance was associated with barbs having thicker cortices and smaller piths (Igic et al. 2018).

Previous work demonstrated that, among black and white feathers, there are varying brightness intensities tied to different feather microstructures, resulting in a brightness gradient (Igic et al. 2018; McCoy et al. 2018). To further address this topic, this study will examine intra- and interspecific comparisons of facial feather microstructure of birds with black (lower reflectance values) and white and gray coloration (higher reflectance values) using reflectance spectroscopy and scanning electron microscopy (Brink and van der Berg 2004; Doucet and Hill 2009; Lee et al. 2009; McCoy et al. 2018).

Objectives

Numerous feather microstructural features might explain the high brightness levels of white feathers (37-70% brightness, Chapter 1), and low brightness of dark feathers (1-10% brightness, Chapter 1). However, few studies have tested these features in relation to their potential tie to adaptations for glare reduction.

This study tested the role of microstructural characteristics in glare reduction (reduction in brightness) adapted feathers. Feather microstructure data were collected on bird species with variation in head plumage coloration that have previously been shown to have reduction in brightness or increased light collecting capabilities (Chapter 1). This study tested the prediction that black

feathers surrounding the eye have distinct microstructural characteristics that differ from those of white or gray feathers that also surround the eye. The following specific hypotheses and predictions were tested.

Feather Microstructure Hypotheses:

H_0: Feather microstructural characteristics will not vary in a consistent pattern that explains reflectance values of black, gray, or white feathers surrounding the eye.

H_1: Black feathers (with low reflectance values) surrounding the eye will have distinct microstructural characteristics that are differentiated from the microstructural characteristics of white or gray plumage (with higher reflectance values). The predictions that supports H_1 are the following:

- Prediction 1: Black feathers will have greater barbule density.
- Prediction 2: Black feathers will have smaller pith:cortex area (μm^2) ratios.

MATERIALS AND METHODS

Feather Microstructure and Spectrometer Data Collection

Specimens: Feather microstructure and plumage brightness was quantified using museum skins of species that exhibit variation in dark facial markings and bill colorations (Eaton and Lanyon 2003; McCoy et al. 2018; Chapter 1). To understand how feather microstructure impacts reduction in brightness properties of feathers a subset of 12 species was chosen from those used for a previous reduction in brightness study (Chapter 1). The species selected represented a range of plumage brightness; from darkest to lightest the species are: Rose-breasted Grosbeak (*Pheucticus ludovicianus,* male), Lesser Scaup (*Aythya affinis,* male), Common Grackle (*Quiscalus quiscula,* male), American Crow, Loggerhead Shrike, Yellow-bellied Sapsucker (*Sphyrapicus varius*, female), Black-crowned Night Heron (*Nycticorax nycticorax*), Forster's Tern (*Sterna forsteri*, breeding adult), Bonaparte's Gull (*Chroicocephalus philadelphia*, non-breeding adult), Herring Gull (*Larus argentatus*), Snowy Egret (*Egretta thula*), and Great Egret (*Ardea alba*) (Table 1). Each species was represented by 2 to 3 specimens, for a total of 35 specimens examined. Care was taken to choose birds that were of the same morph as the original specimen of each species to

ensure plumage color and patterns would be similar among all sample birds of the same species.

Table 1. Average reduction in brightness and average head brightness for the 12 species used in this study, listed from darkest to lightest. The averages were calculated from all head tilts at the 0° and 45° head rotations from Chapter 1, the 90° head rotation was excluded because of its lack of significance.

Species Name	Average Head Brightness (%)	Reduction in Rrightness (%)
Rose-Breasted Grosbeak	4.078	0.285
Lesser Scaup	4.785	0.116
Common Grackle	5.819	0.944
American Crow	7.250	2.483
Loggerhead Shrike	13.441	0.719
Yellow-bellied Sapsucker	14.950	-0.075
Black-crowned Night Heron	18.888	-1.015
Forster's Tern	19.872	-0.068
Bonaparte's Gull	40.188	-1.969
Herring Gull	43.388	-1.502
Snowy Egret	45.035	-0.206
Great Egret	56.400	-2.067

Plumage Brightness: Plumage brightness was measured for each specimen using reflectance spectroscopy which measures the percentage of light reflected by a sample, and is expressed as percent reflection (or brightness percentage) (Gomez and Théry 2007; Santos et al. 2007; Doucet and Hill 2009; McCoy et al. 2018). Black or dark plumage can express reflectance values below 10% (Doucet and Hill 2009; Ismar et al. 2014; McCoy et al. 2018; Fig. 2). White plumage can express reflectance values as high as 50-70% (Stuart-Fox et al.

2018; Fig. 2). These measurements were taken using a USB 2000 Ocean Optics spectrometer and DH-mini deuterium-halogen lamp (Ocean Optics) following standard protocols (Gomez and Théry 2007; Santos et al. 2007). A bifurcated optic cable (R400-7-UV/VIS Ocean Optics) used light from the lamp for illumination and a probe encased in a rubber stopper was held at 90° from the sample surface (Mennill et al. 2003). Prior to measuring samples, baseline readings were established using a white standard (Spectralon Diffuse Reflectance Standard, Labsphere) and by shuttering the lamp (Stavenga and Wilts 2014). Reflectance data were collected with OceanView (Ocean Optics) software with 10 scans averaged across a single reading. Reflectance values (percentages) were calculated from the spectrum of 300nm-700nm to represent the average avian visual spectrum (Pearn et al. 2003; Mays et al. 2006; Hofmann et al. 2007; Avilés et al. 2011; Pascual et al. 2014; Stavenga and Wilts 2014).

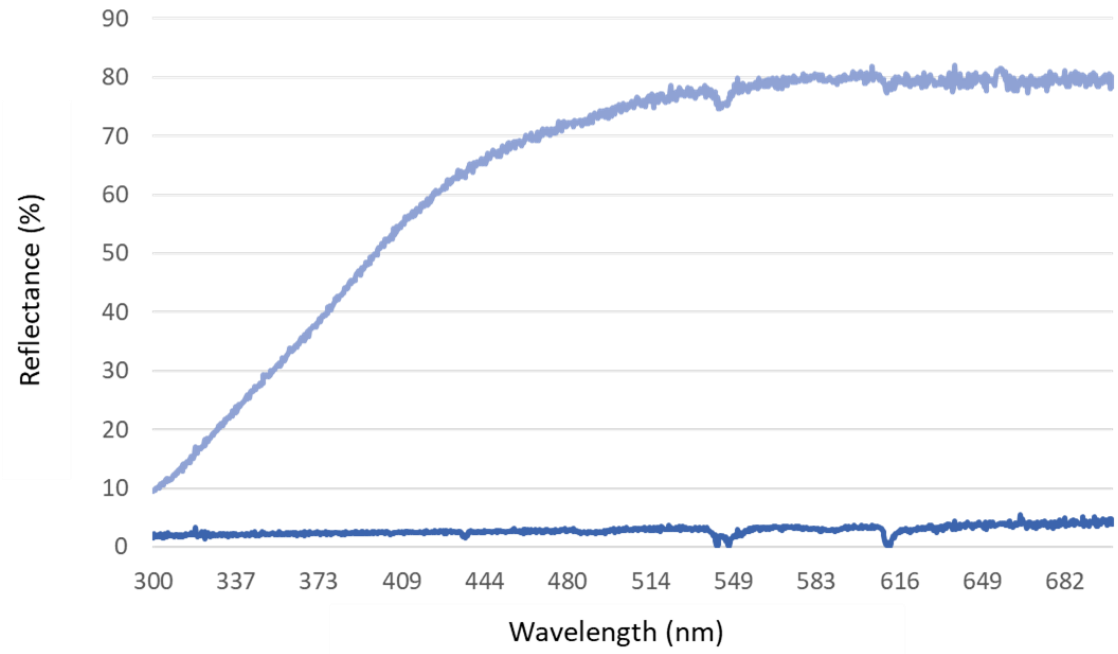


Figure 2. Example reflectance spectra of a white bird (Great Egret, light blue) and a dark bird (Rose-breasted Grosbeak, dark blue).

Plumage was categorized and examined by dividing the head of each specimen was into four quarters (Quarter 1: dorsal and anterior to the eye; Quarter 2: ventral and anterior to the eye; Quarter 3: ventral and posterior to the eye; Quarter 4: dorsal and posterior to the eye; Fig. 3) with the eye as center point. Each patch within a quarter underwent three scans for reflectance values, using the methods described above, and were averaged within each quarter. Reflectance values for each quarter were calculated by averaging the reflectance values of all patches in that quarter.

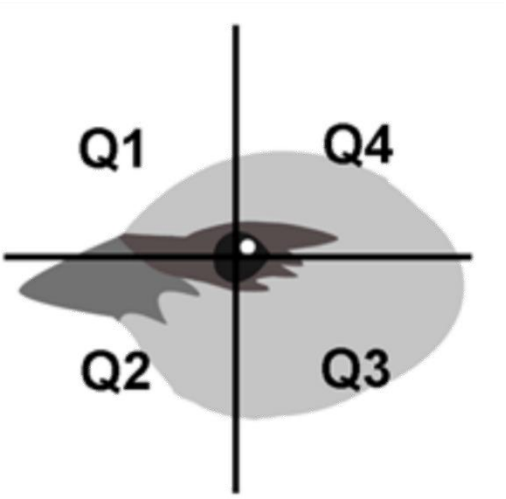


Figure 3. Representation of a specimen with regions divided into quarters for measuring patch reflectance. Every patch in a quarter had its reflectance measured and then an average reflectance from all patches was calculated for each quarter.

Feather Samples: Pith and cortex thickness of the barb ramus and barbule density were quantified in this study as these structural characteristics are correlated with plumage brightness across several species (Lei et al. 2002. Shawkey et al. 2005; Galván 2011; Stuart-Fox et al. 2018). The feathers closest to the eye were considered most relevant to this study, so all samples were plucked within a half centimeter radius from the center of the eye. For each specimen two feathers were taken from each color patch represented within a quarter. The two feathers are taken as close to each other as possible to ensure maximum similarities between them in size, shape, and color. Birds were placed under a dissecting scope and feathers were carefully removed from each specimen using forceps. One feather per specimen, used for barbule counts, was applied directly to an aluminum stub (Varricchio and Jackson 2004). The other

feather, used for pith and cortex measurements, was cut midway down the lengths of several barbs using a razor under the dissecting scope before being adhered to an aluminum stub (Galván 2011; Igic et al. 2018). Samples were then sputter coated for 300 seconds using gold-palladium (Aire 1982; Klann et al. 2009).

Scanning Electron Microscopy: Feather microstructure was examined using a Hitachi S-2300 scanning electron microscope (SEM) operating at 8-10 kV (Brink and van der Berg 2004; Moreno and Meseguer 2008). Magnifications between 40X-400X were used to capture micrographs for barbule counts, and magnifications upwards of 500X were used to capture clear micrographs of the pith and cortex (Brink and van der Berg 2004). Micrographs were taken using Quartz PCI (Hitachi High Technologies, America, Pleasanton, CA), which provides a scale bar for each micrograph and records magnification. Micrographs taken for barbule counts had many barbs visible while maintaining clarity of individual barbules. For pith and cortex area measurements, micrographs were taken of a single barb randomly chosen from those cut on the sample feather (Fig. 4; Galván 2011).

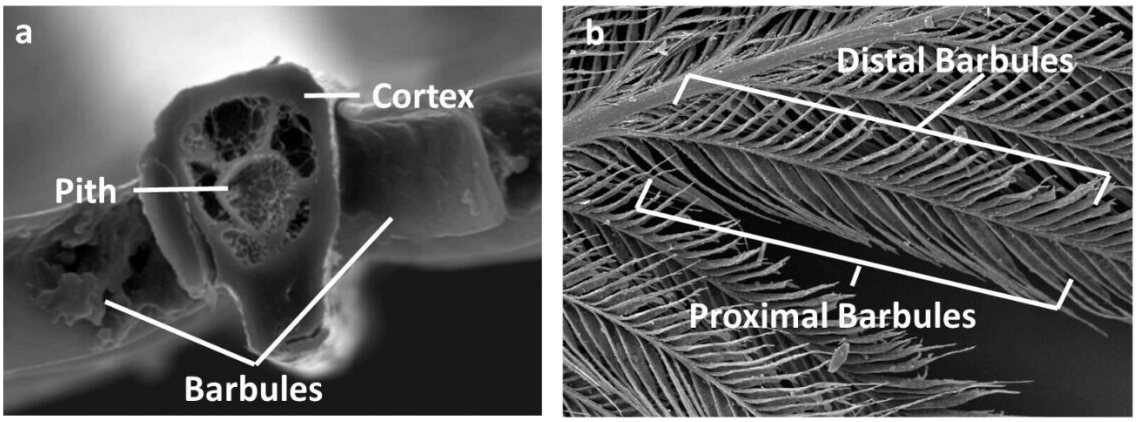


Figure 4. Examples of micrographs of feather microstructure taken with the SEM. **a.** The inner layers of a barb ramus are visible after being cut midway down the barb. **b.** Distal and proximal sides of a feather barb and barbules are labeled.

Microstructural Features: Micrographs were uploaded into ImageJ v. 1.52a (National Institute of Health) for barbule density quantification and pith and cortex measurements (Rasband 2018). To determine barbule density, three barbs were randomly chosen from each sample, and all distal and proximal barbules were counted within 500 µm from the base of each barb (Galván 2011; D'Alba et al. 2014; Fig. 4b). Average barbule density was calculated as the mean sum of proximal and distal barbules for each barb (de Zwaan et al. 2017). The measurements of proximal, distal, and average barbule density were then averaged across the three barbs to calculate a single average for each sample (Galván 2011). Pith and cortex area (μm^2) were found for each sample using the polygon selection and measuring tools in ImageJ (Rasband 2018). A pith:cortex ratio was calculated for each sample using the pith and cortex areas; a smaller pith:cortex ratio is representative of a smaller pith with a thicker cortex, and a

larger pith:cortex ratio is representative of a larger pith with a thinner cortex (Galván 2011; Fig. 5)

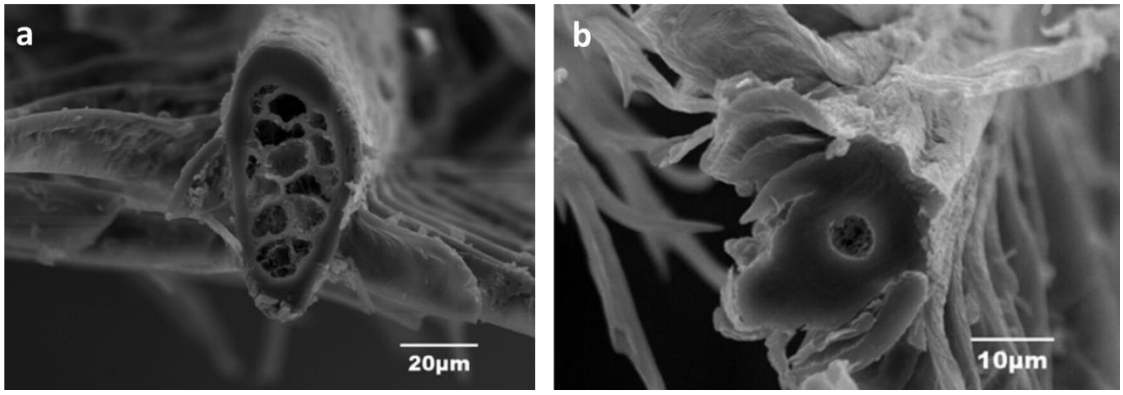


Figure 5. Examples of barb rami with larger (a) and a smaller (b) pith:cortex ratio.

Statistical Methods:

For the purpose of examining the relationship between feather microstructure and feather brightness stepwise regressions were used in JMP v.14 (SAS Institute Inc., Cary, NC, 1989-2007; Shawkey et al. 2003; Doucet et al. 2005; Griggio et al. 2010). Stepwise regression models established which predictor variables (distal barbule density, proximal barbule density, and pith:cortex ratio) best predicted changes in the response variable (plumage brightness %) (Shawkey et al. 2003; Doucet et al. 2005). The models were developed using JMP's P-value threshold option with the forward stepwise addition of predictor variables with a maximum p-value of 0.25 (the default) to enter the model (Griggio et al. 2010). Goodness of fit of the models was, in part,

determined by the multiple regression adjusted r-squared (R^2 Adj), in which r-squared is adjusted for using multiple rather than a single predictor variable (St-Louis et al. 2006). Predictor variable importance was found through t-tests, and deemed significant at α-values below 0.05 (Mills et al. 1991). In order to meet model assumptions of normality the response variable (brightness %) was square root transformed, while the predictor variables remained untransformed (Mills et al. 1991; Griggio et al. 2010). Effects of predictors in the final regressions are shown with leverage plots because multiple predictors were chosen for the models. A leverage plot displays how the addition of one predictor variable of interest affects the model with the other predictor variables already included in the model (Sall et al. 2017). The plots were based on two sets of residuals, one set from regressing the added predictor of interest on the other predictors already in the model and the other set from regressing the response on the other predictors already in the model. Then, the first set of residuals was added to the mean of the predictor of interest and these were plotted along the X-axis, while the second set of residuals were added to the mean of the response and these are plotted along the Y-axis (Sall et al. 2017). The correlations and relationships between the predictor variables (distal barbule density, proximal barbule density, and pith:cortex ratio) of average head brightness were examined using principal component analyses (PCA; Sodhi et al. 1999; Robinson-Wolrath and Owens 2003). PCAs use the relationships

between predictor variables to explain covariance among them with eigenvalues and eigenvectors (Sodhi et al. 1999; Robinson-Wolrath and Owens 2003).

RESULTS

Plumage Brightness:

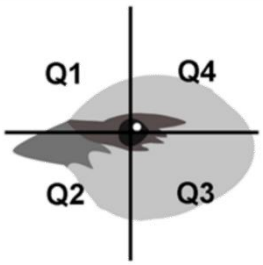
Brightness values were low for species with overall dark plumage across all four quarters (Table 2). This includes the American Crow, Common Grackle, Lesser Scaup and Rose-breasted Grosbeak. Each of these species had average brightness values below 10%, except for the American Crow, which had one specimen where brightness in Quarter 2 was slightly above 10% (Table 2).

Species with overall lighter heads saw more variation in brightness values than species with mostly darker plumage. The species with the highest brightness values were Great Egrets and Snowy Egrets, while the Herring Gull had more variation between its specimens (Table 2). Lastly, the Bonaparte's Gull makes up the lower end of brightness values for the species with overall lighter heads (Table 2).

Species with both light and dark plumage had the greatest amount of variation in plumage brightness values. The Black-crowned Night Heron exhibited the most variation of these species (Table 2). For the Forster's Tern plumage dorsal to the eye (Quarters 1 and 4) was typically darker in this species, while plumage ventral to the eye (Quarters 2 and 3) was lighter (Table 2). For the

Loggerhead Shrike plumage ventral to the eye (Quarters 2 and 3) was brighter and plumage dorsal to the eye had lower brightness values (Table 2). The Yellow-bellied Sapsucker had the least amount of variation in average head brightness (Table 2). Plumage anterior and dorsal to the eye (Quarter 1) was darkest for this species while the other facial regions (Quarters 2, 3, and 4) were somewhat brighter (Table 2).

Table 2. Plumage brightness (%) for each species and specimen used in this study. Average head brightness is the average taken from all four quarters.



Species Name	Average Head Brightness (%)	Q1 Brightness (%)	Q2 Brightness (%)	Q3 Brightness (%)	Q4 Brightness (%)
American Crow 1	7.250	9.077	13.471	4.149	2.304
American Crow 2	2.917	2.612	1.814	3.560	3.681
American Crow 3	2.624	2.919	1.590	3.219	2.769
Black-crowned Night Heron 1	18.888	12.963	25.085	32.931	4.571
Black-crowned Night Heron 2	38.863	59.129	50.491	42.473	3.359
Black-crowned Night Heron 3	41.877	58.116	51.226	50.030	8.136
Bonaparte's Gull 1	40.188	56.681	28.530	35.295	40.246
Bonaparte's Gull 2	39.700	58.327	32.080	31.501	36.890
Bonaparte's Gull 3	38.091	41.812	36.994	37.943	35.614
Common Grackle 1	5.819	6.705	8.672	4.824	3.076
Common Grackle 2	3.734	1.392	3.057	4.504	5.983
Common Grackle 3	4.453	5.452	2.052	5.573	4.736
Forster's Tern 1	19.872	17.371	28.312	30.929	2.876
Forster's Tern 2	16.379	3.396	25.692	33.068	3.360
Forster's Tern 3	15.314	2.918	26.060	29.066	3.212
Great Egret 1	56.400	61.289	58.918	48.295	57.097
Great Egret 2	62.507	63.359	64.706	64.832	57.131
Great Egret 3	60.945	59.759	71.234	58.790	53.998

Table 2, continued.					
Herring Gull 1	43.388	50.495	35.085	47.420	40.550
Herring Gull 2	51.086	53.921	51.481	45.950	52.993
Herring Gull 3	37.074	46.248	28.980	30.743	42.324
Lesser Scaup 1	4.785	4.704	6.830	4.377	3.232
Lesser Scaup 2	2.760	3.216	1.940	3.305	2.581
Lesser Scaup 3	3.471	4.277	3.518	2.873	3.215
Loggerhead Shrike 1	13.441	9.995	13.065	22.039	8.666
Loggerhead Shrike 2	15.755	9.959	18.836	25.820	8.406
Rose-Breasted Grosbeak 1	4.078	3.454	5.273	5.157	2.426
Rose-Breasted Grosbeak 2	3.876	3.116	4.589	4.639	3.158
Rose-Breasted Grosbeak 3	3.393	2.015	4.406	3.942	3.208
Snowy Egret 1	45.035	39.713	40.397	48.135	51.895
Snowy Egret 2	43.112	40.763	41.357	39.249	51.080
Snowy Egret 3	50.263	49.168	47.278	52.145	52.463
Yellow Bellied Sapsucker 1	14.950	5.156	19.376	18.326	16.944
Yellow Bellied Sapsucker 2	15.852	4.083	25.601	20.908	12.817
Yellow Bellied Sapsucker 3	15.032	3.460	24.072	20.298	12.299

Feather Microstructure:

For each quarter, feathers were measured for pith:cortex ratio and distal and proximal densities. For the whole head, the values were averaged and the pith:cortex ratio and barbule density ranged widely across the different specimens (Table 3). For the pith:cortex ratio, the Great Egret and Snowy Egret had low values (Table 3), On the other hand darker species such as the Rose-breast Grosbeak and Common Grackle had higher average values (Table 3). Both distal and proximal barbule density also varied among the different species (Table 3). With average distal barbule density, lighter colored species like the Bonaparte's Gull and Herring Gull had more distal barbules per 500 µm than darker species such as the American Crow and Lesser Scaup (Table 3). For

proximal barbule density these same species had similar amounts of barbules per 500 µm (Table 3).

Table 3. Average pith:cortex ratios and barbule densities for each species.

Species Name	Average Pith:Cortex Ratio	Average Distal Barbule Density	Average Proximal Barbule Density
American Crow 1	0.212	15.167	13.417
American Crow 2	0.124	16.500	12.917
American Crow 3	0.162	16.250	14.583
Black-crowned Night Heron 1	0.077	17.000	15.000
Black-crowned Night Heron 2	0.085	17.833	14.750
Black-crowned Night Heron 3	0.122	18.000	14.917
Bonaparte's Gull 1	0.216	19.125	15.000
Bonaparte's Gull 2	0.137	17.167	13.458
Bonaparte's Gull 3	0.115	19.208	14.375
Common Grackle 1	0.332	18.250	14.917
Common Grackle 2	0.392	18.750	15.333
Common Grackle 3	0.264	19.667	15.917
Forster's Tern 1	0.301	26.208	18.833
Forster's Tern 2	0.260	22.917	19.750
Forster's Tern 3	0.123	21.833	17.542
Great Egret 1	0.047	18.833	14.167
Great Egret 2	0.031	19.833	15.667
Great Egret 3	0.019	18.833	14.667
Herring Gull 1	0.157	19.667	14.333
Herring Gull 2	0.078	20.750	16.083
Herring Gull 3	0.070	19.750	15.083
Lesser Scaup 1	0.216	16.000	14.583
Lesser Scaup 2	0.172	15.500	13.750
Lesser Scaup 3	0.221	17.250	14.083
Loggerhead Shrike 1	0.398	19.167	15.167
Loggerhead Shrike 2	0.433	19.917	17.125
Rose-Breasted Grosbeak 1	0.467	15.417	13.250
Rose-Breasted Grosbeak 2	0.374	20.083	17.333
Rose-Breasted Grosbeak 3	0.331	20.250	15.167
Snowy Egret 1	0.051	23.333	16.417
Snowy Egret 2	0.000	20.500	15.167
Snowy Egret 3	0.009	22.083	15.750

Table 3, continued.			
Yellow Bellied Sapsucker 1	0.027	21.667	17.667
Yellow Bellied Sapsucker 2	0.010	18.583	15.042
Yellow Bellied Sapsucker 3	0.037	20.625	17.417

Plumage Brightness Relationship to Feather Structure:

Average barbule density is a simple linear combination of proximal and distal barbules densities and was found to be redundant when running stepwise regressions in models that included distal and proximal barbules densities. Because it overparameterized the models, average barbules density was excluded when reporting the results.

Table 4. Stepwise regression results for feather microstructure explaining plumage brightness (square-root transformed) by plumage location. If an effect's p-value for entry exceeded 0.25, that effect was not included in the final model but it's entry p-value is listed in the table.

	Regression Effects **Estimate** **(SE)** ***P*-values**				**Regression Statistics**	
Plumage Location	**Intercept**	**Pith:Cortex Ratio**	**Distal Barbule Density**	**Proximal Barbule Density**	**Adjusted R^2**	**RMSE**
Q1 estimate	8.261	-9.370	0.255	-0.456	0.359	2.058
Q1 S.E.	2.477	2.381	0.165	0.232		
Q1 *P*-value	0.002	0.0004	0.131	0.058		
Q2 estimate	3.850	-9.034	0.275	-0.218	0.258	1.860
Q2 S.E.	1.493	2.600	0.159	0.207		
Q2 *P*-value	0.014	0.001	0.094	0.300		
Q3 estimate	2.004	-6.886	0.361	-0.179	0.261	1.815
Q3 S.E.	2.572	2.217	0.198	0.259		
Q3 *P*-value	0.441	0.004	0.077	0.492		
Q4 estimate	-0.820	-5.545	0.584	-0.348	0.509	1.644
Q4 S.E.	1.944	1.975	0.197	0.282		

Table 4, continued.						
Q4 *P*-value	0.675	0.008	0.005	0.226		
Average estimate	4.367	-7.824	0.659	-0.730	0.506	1.504
Average S.E.	2.556	2.002	0.201	0.310		
Average *P*-value	0.097	0.0005	0.003	0.025		

Relationship to Pith:Cortex Ratio:

Feather brightness anterior to the eye (Quarters 1 and 2) was affected by the pith:cortex ratio (R^2 Adj=0.36, RMSE=2.06, *P*=<0.001; R^2 Adj=0.26, RMSE=1.86, *P*=0.001; respectively). A smaller pith:cortex ratio (little to no pith, thicker cortex) is associated with brighter plumage and as the pith:cortex ratio increased (larger pith and thinner cortex) plumage got darker (Fig. 6a).

Plumage brightness for feathers posterior to the eye (Quarters 3 and 4) was also affected by the pith:cortex ratio (R^2 Adj=0.26, RMSE=1.82, *P*-value=0.004; R^2 Adj=0.51, RMSE=1.64, *P*-value=0.008; respectively). Similar to plumage anterior to the eye the pith:cortex ratio was negatively associated with plumage brightness, meaning larger piths and thinner cortices were correlated with darker plumage (Fig. 6b).

For the entire head average feather brightness was significantly affected by the pith:cortex ratio (R^2 Adj=0.51, RMSE=1.50, *P*-value=<0.001). The pith:cortex ratio was negatively correlated with feather brightness, which means darker plumage was driven by larger piths and thinner cortices and brighter plumage was associated with the opposite patterns (Fig. 6c).

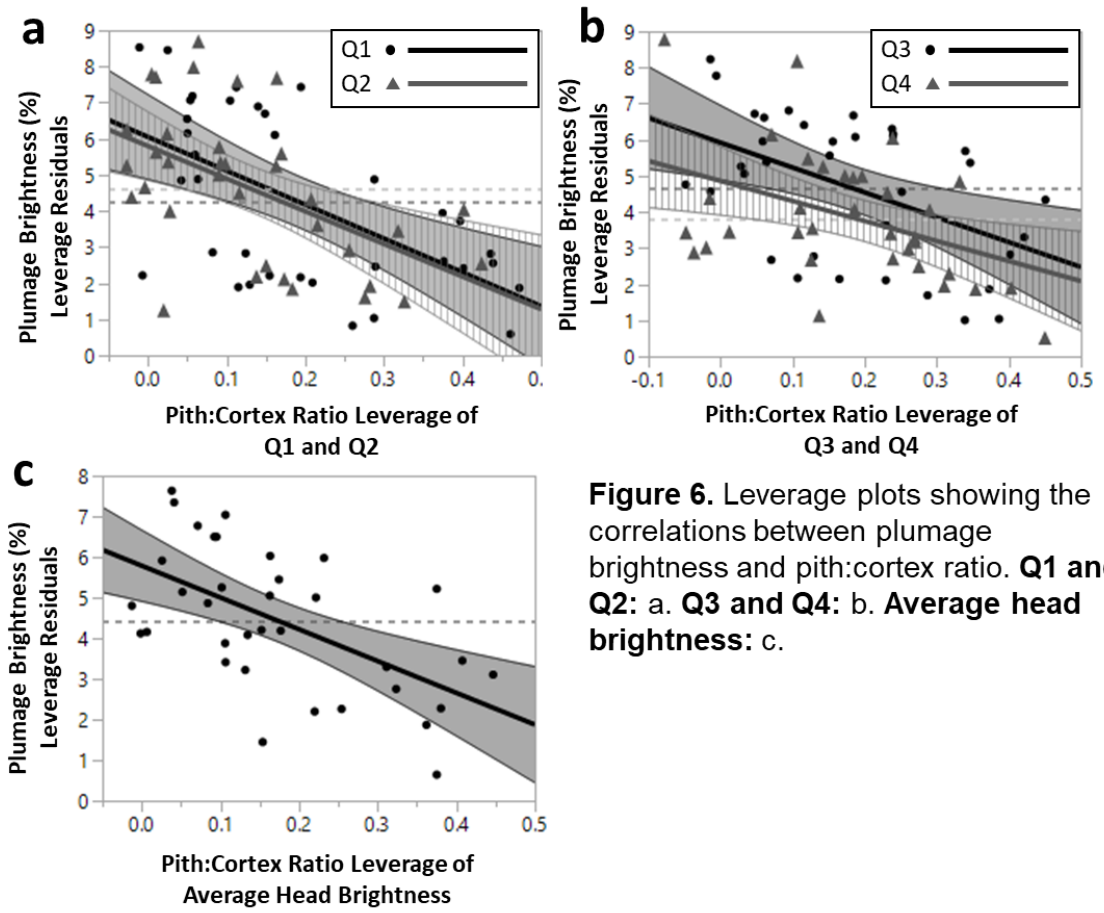


Figure 6. Leverage plots showing the correlations between plumage brightness and pith:cortex ratio. **Q1 and Q2:** a. **Q3 and Q4:** b. **Average head brightness:** c.

Relationship to Barbule Density:

Barbule density displays varying patterns between plumage anterior and dorsal to the eye (Quarter 1) and plumage anterior and ventral to the eye (Quarter 2). Neither proximal or distal barbule density had a significant correlation with plumage brightness in feathers dorsal and anterior to eye (Quarter 1). For plumage brightness in feathers anterior and ventral to eye (Quarter 2), only distal barbule density had a slight positive trend, indicating

increased distal barbule density was associated with brighter plumage (R^2 Adj=0.26, RMSE=1.86, *P*-value=0.09; Fig. 7b). Proximal barbule density seems to have no effect on plumage brightness in this region (R^2 Adj=0.26, RMSE=1.86, *P*-value=0.30; Fig. 7a).

No plumage posterior to the eye had any significant associations between brightness and proximal barbule density (Q3: R^2 Adj=0.26, RMSE=1.82, *P*-value=0.49; Q4: R^2 Adj=0.51, RMSE=1.64, *P*-value=0.23; Fig. 7c). For feathers posterior and dorsal to the eye (Quarter 4) distal barbule density had a significant positive correlation with plumage brightness, indicating that more densely packed distal barbules were associated with increased brightness (R^2 Adj=0.51, RMSE=1.64, *P*-value=0.01; Fig. 7d). Plumage brightness for feathers posterior and ventral to eye (Quarter 3) did not have a significant relationship with distal barbule density (R^2 Adj=0.26, RMSE=1.82, *P*-value=0.08; Fig. 7d).

Average plumage brightness of the entire head was significantly impacted by distal and proximal barbule density in different ways despite the positive correlation between distal and proximal barbule density. Proximal barbule density was negatively correlated with feather brightness (R^2 Adj=0.51, RMSE=1.50, *P*-value=0.03; Fig. 7e). In contrast, distal barbule density was positively correlated with feather brightness, indicating that as distal barbule density increased feather brightness did as well (R^2 Adj=0.51, RMSE=1.50, *P*-value=0.002, Fig. 7f).

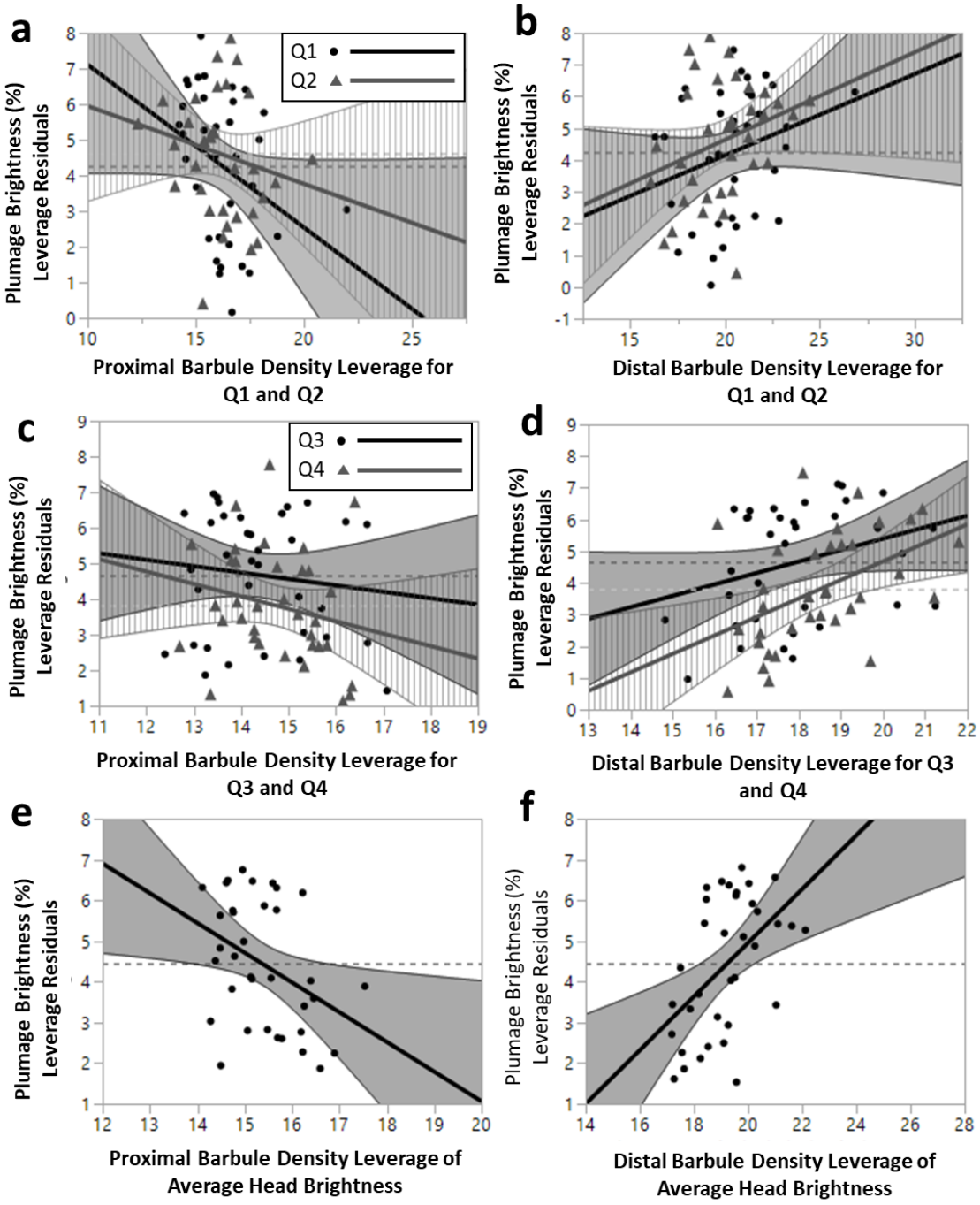


Figure 7. Leverage plots of the associations between plumage brightness and proximal and distal barbule density. **Q1 and Q2:** a and b. **Q3 and Q4:** c and d. **Average Head Brightness:** e and f.

Principal Component Analysis:

To gain an understanding of their relationship independent of head brightness the three microstructural features are broken up into two principal components, barbule density (PC1) and pith:cortex ratio (PC2), that explain 95% of the variance among the predictor variables (Tables 5, 6). Proximal and distal barbule density are correlated, but not with pith:cortex ratio (Table 7). Therefore, pith:cortex ratio and barbule density are largely unrelated and did not influence each other.

PC1 is largely influenced by both proximal and distal barbule density, but the amount of influence pith:cortex ratio had on this principal component was negligible (Table 5). On the other hand, PC2 was almost solely influenced by the pith:cortex ratio and had little impact from proximal or distal barbule density (Table 5). The absence of a relationship between barbule density and pith:cortex ratio is also evident in the correlation matrix; proximal and distal barbule density were highly correlated, while pith:cortex ratio was not correlated with proximal or distal barbule density (Table 6). The lack of association between barbule density and the pith:cortex ratio is illustrated in PCA plot (Fig. 8), wherein the perpendicular relationship between the two indicates no correlation.

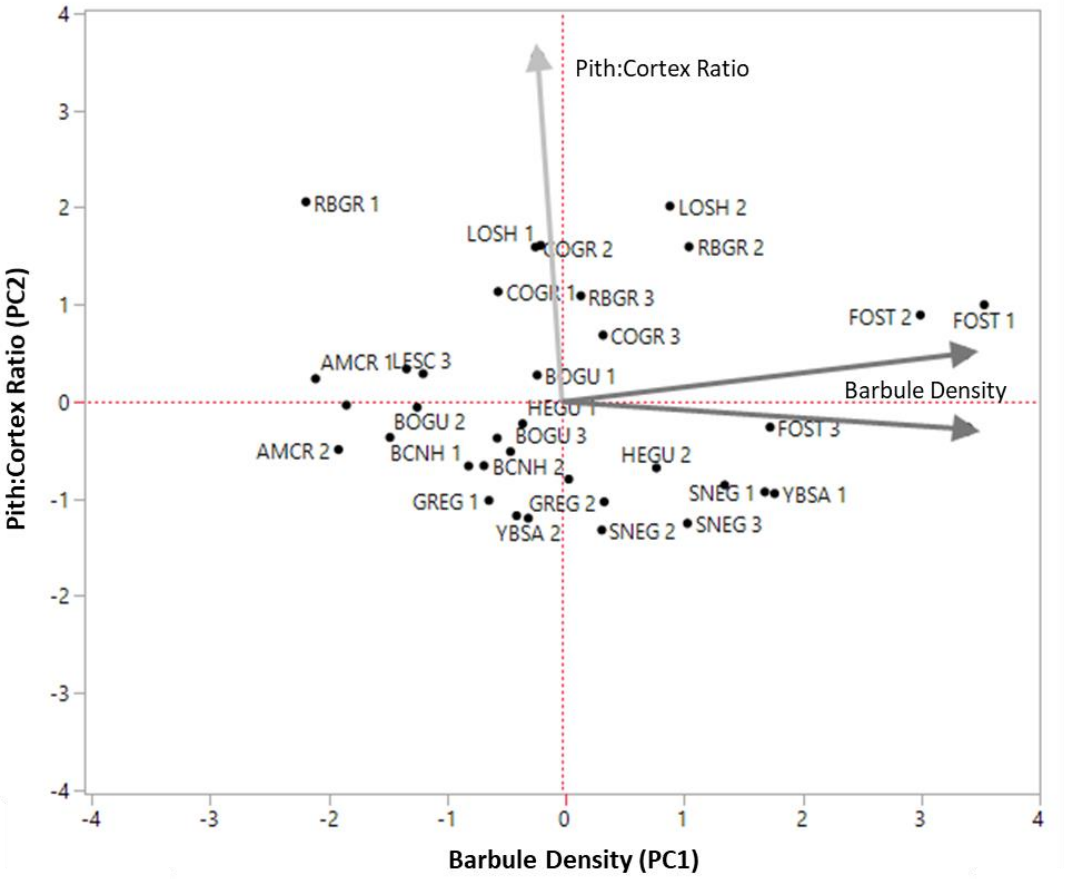


Figure 8. PCA plot; score indicates how a particular observation weighs on a particular eigenvector. Principal component score is calculated by multiplying the observations' predictor values by the principal component eigenvectors. Darker species are located towards the upper left closer to the positive pith:cortex ratio vector, while lighter species congregated more in the lower right, and species with both light and dark plumage are generally grouped between the two. See Appendix A for bird species alpha codes.

Table 5. Eigenvalues of plumage brightness predictor variables for the entire head.

Entire Head PC Number	Eigenvalue	Variance (%)	Cumulative Variance (%)
1	1.832	61.066	61.066
2	1.019	33.966	95.032

Table 6. Eigenvectors of plumage brightness predictor variables. Bold text indicates which variables had a major influence on each principal component.

	PC1	PC2
Average Pith:Cortex Ratio	-0.042	**0.987**
Average Distal Barbule Density	**0.708**	-0.078
Average Proximal Barbule Density	**0.704**	0.138

Table 7. Correlation matrix for plumage brightness predictor variables. Correlations range from 1 to -1, with numbers closer to 1 or -1 indicating a strong correlation and numbers closer to 0 indicating little to no correlation. Bold numbers indicate significance.

	Average Pith:Cortex Ratio	Average Distal Barbule Density	Average Proximal Barbule Density
Average Pith:Cortex Ratio	**1**	-0.118	0.068
Average Distal Barbule Density		**1**	**0.830**
Average Proximal Barbule Density			**1**

DISCUSSION

Feather microstructure appears to affect feather brightness, although not in the ways that I had initially predicted. Overall, the data indicates smaller piths and larger cortices (smaller pith:cortex ratios) are associated with brighter feathers rather than darker ones. Additionally, the relationship of barbule density to feather brightness was not straight-forward. When examined individually distal and proximal barbule densities display opposing trends in regards to feather brightness, despite being correlated (Fig. 7, Table 5). Distal and proximal barbules can possess differing structural features, regardless of branching off the same ramus, including that distal barbules have hooklets used to interlock with the proximal barbules of the neighboring barb (Prum 1990; Dove and Koch 2011; Harvey et al. 2013). Future studies could explore the relationship and differences between proximal and distal barbule density and barbule microstructure and its effect on feather coloration.

Darker colored plumage was positively associated with thinner cortices, larger piths, decreased distal barbule density for some facial regions, and increased proximal barbule density for other facial regions. Much of these results are contrary to previous work that correlates black feathers with thicker cortices, smaller piths, and overall increased barbule density (Galván 2011; D'Alba et al.

2014). Great Tits, Black-capped Chickadees, and Zebra Finches all saw an increase in the darkness of their plumage driven by the above features (Galván 2011; D'Alba et al. 2014). The only prediction of this study supported in the results is the association of increased proximal barbule density with darker plumage.

Varying pith and cortex sizes seem to drive plumage brightness levels in all facial feathers. Across 12 species darker plumage was correlated with thinner cortices and larger piths, while brighter plumage typically had thicker cortices and smaller piths. The patterns of pith and cortex size found in this study more closely mirror the findings of Igic et al. (2018), and other research (Stuart-Fox et al. 2018) that found brighter white plumage had thicker cortices and smaller piths. These results also support the conclusion that a smaller pith:cortex ratio aids in the incoherent scattering of light to give feathers a white appearance (Dyck 1979; Igic et al. 2018; Stuart-Fox et al. 2018).

Of the 12 species examined here all the light-colored birds saw increased light entering the eye in a reduction in brightness study versus all of the darker birds which had enjoyed increased reduction in brightness (Chapter 1). Reduction in brightness for the birds in the group with both light and dark plumage was varied, one species (Loggerhead Shrike) saw increased reduction in brightness while the other three (Black-crowned Night Heron, Forster's Tern, and Yellow-bellied Sapsucker) did not. Although the amount of reduction in brightness gained by darker plumage is not large, it might still be useful for birds

in predator detection or increased foraging efficiency (Rohwer et al. 1983; Martin 2007). Interestingly, Igic et al. (2018) also found that smaller birds had less bright white feathers, and if this trend applies to facial feathers perhaps less light is bounced into the eye.

This study demonstrates the importance of distinguishing between effects of proximal and distal barbules. Despite the strong positive correlation between proximal and distal barbule density, these two aspects of feather microstructure may affect feather reflectance in opposite ways in different regions of the head (Galván et al. 2009; Shawkey et al. 2011). The only area of the head with a clear relationship between brightness and barbule density was plumage dorsal and posterior to the eye (Quarter 4). In this location increased brightness was driven by an increase in distal barbule density. Data from other head regions indicate possible trends that fall just short of statistical significance. Brightness in plumage ventral to the eye (Quarters 2 and 3) displayed a pattern similar to that seen in quarter 4; increased plumage brightness is indicated with increased distal barbule density. In plumage dorsal and anterior to the eye (Quarter 1), there is a trend for increased proximal feather density being associated with darker feathers.

Continued research into this topic could explore how other microstructural features impact glare reduction. Melanin granule arrangement, barbule shape, ramus shape, and several other features have been shown to impact feather reflectance (Lee et al. 2009, 2010; Igic et al. 2018). Within species variation of

these characteristics can also result in plumage differences between the sexes and be indictive of the health of a bird (Doucet 2002; Lee et al. 2009; D'Alba et al. 2014). Future studies into the relationship between glare reduction and plumage brightness could also investigate the roles of bird sex and health. It might be that healthier birds reap more substantial benefits of glare reduction by having a more optimal feather microstructure. The advantages conferred by increased glare reduction might enable these healthier birds to better pass on their genes, thereby driving the species towards greater glare reduction. Ultimately, combining examinations of these microstructural features, measuring feather reflectance, and glare reduction tests can reveal important underlying relationships between these factors.

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APPENDICES

Appendix A. List of specimens used in this study, with species alpha codes, sex, molt, presence or not of face mask, and if it was used in the bill index.

Specimen Information					
Species common name	**Species Alpha Code**	**Sex (M/F/U)**	**Molt: Breeding (B) or Nonbreeding (NB)**	**Face Mask (Yes/No)**	**Used in Bill Index (Yes/No)**
American Avocet	AMAV	M	NB	No	Yes
American Crow	AMCR	U	NB	Yes	Yes
Black-crowned Night Heron	BCNH	U	NB	Yes	Yes
Blue Jay	BLJA	U	NB	No	Yes
Blue-winged Teal	BWTE	M	NB	No	Yes
Bonaparte's Gull	BOGU	M	NB	No	Yes
Brown-headed Cowbird	BHCO	M	NB	Yes	Yes
Common Grackle	COGR	M	NB	Yes	Yes
Eastern Meadowlark	EAME	U	B	No	Yes
Forster's Tern	FOST	U	B	Yes	No
Great Egret	GREG	U	NB	No	No
Green Heron	GRHE	U	NB	Yes	Yes
Green-winged Teal	GWTE	M	B	No	Yes
Hawk sp.	HAWK sp.	U	NB	No	Yes
Herring Gull	HEGU	F	NB	No	Yes
Killdeer	KILL	U	NB	No	Yes
Lesser Scaup	LESC	M	B	Yes	Yes
Little Blue Heron	LBHE	U	NB	Yes	No
Loggerhead Shrike	LOSH	M	NB	Yes	Yes

Appendix A, continued.

Mallard	MALL F	F	NB	No	No
Mallard	MALL M	M	B	No	Yes
Northern Cardinal	NOCA F	F	NB	Yes	Yes
Northern Cardinal	NOCA M	M	NB	Yes	Yes
Northern Flicker	NOFL	M	NB	No	Yes
Piping Plover	PIPL	F	NB	No	No
Purple Martin	PUMA	M	NB	Yes	Yes
Red-bellied Woodpecker	RBWO F	F	NB	No	Yes
Red-bellied Woodpecker	RBWO M	M	NB	No	Yes
Red-winged Blackbird	RWBL	M	NB	Yes	Yes
Ring-billed Gull	RBGU	U	NB	No	Yes
Rose-breasted Grosbeak	RBGR	M	B	No	Yes
Sanderling	SAND	F	NB	No	Yes
Snowy Egret	SNEG	U	NB	No	No
Thrush sp.	THRUSH	U	NB	No	Yes
Wood Duck	WODU F	F	NB	No	No
Wood Duck	WODU M	M	B	Yes	No
Yellow-bellied Sapsucker	YBSA	F	NB	Yes	No

Appendix B. List of average reduction in brightness percentages for all species examined in this study, at each head rotation and head tilt.

	Reduction in Brightness (%)								
	0° Head Rotation			45° Head Rotation			90° Head Rotation		
Species common name	Head Tilts			Head Tilts			Head Tilts		
	45°	90°	135°	45°	90°	135°	45°	90°	135°
American Avocet	-1.24	-1.40	-1.67	-0.49	0.81	0.76	-0.76	-3.82	-3.79
American Crow	-0.74	0.83	1.41	1.84	5.80	5.77	-4.34	-4.86	-10.26
Black-crowned Night Heron	-0.54	-0.95	-0.72	-1.35	-1.53	-1.01	-1.57	2.58	-0.34
Blue Jay	-1.24	-1.25	-1.17	1.01	-2.52	-1.64	5.28	0.23	-1.13
Blue-winged Teal	-0.87	0.10	-0.51	-0.61	0.41	-0.33	0.01	2.49	1.09
Bonaparte's Gull	-2.24	-2.14	-1.52	-1.58	-4.61	0.27	-4.53	-0.45	7.58
Brown-headed Cowbird	-0.52	-0.40	0.11	0.55	-0.10	0.59	-0.46	-0.19	-0.41
Common Grackle	-0.88	-0.75	-0.16	2.20	2.94	2.31	3.19	2.34	1.42
Eastern Meadowlark	-1.5	-1.70	-1.52	0.23	1.97	-0.52	3.41	1.56	1.63
Forster's Tern	-0.63	-0.06	1.01	-0.68	0.26	-0.30	1.33	-0.53	1.18
Great Egret	-0.89	0.02	-1.59	-2.00	-3.20	-4.75	14.03	2.65	2.35
Green Heron	-0.91	0.03	0.00	0.24	-2.09	-1.04	0.55	-1.13	-0.91
Green-winged Teal	-0.39	-0.30	-0.12	2.36	-0.78	8.08	3.27	2.99	0.78
Hawk sp.	-1.06	-0.83	-0.96	0.87	-1.61	-1.95	3.96	0.15	0.33
Herring Gull	-2.13	-1.75	-2.13	0.88	-1.71	-2.17	2.57	1.41	2.14
Killdeer	-1.47	-1.00	-0.72	11.39	2.67	-1.47	-0.87	7.57	0.88
Lesser Scaup	-0.81	0.10	-1.01	2.04	0.76	-0.38	27.65	18.12	9.54
Little Blue Heron	-0.17	0.20	0.84	0.99	2.11	-2.28	0.69	0.50	-0.33
Loggerhead Shrike	0.4	-1.16	0.40	4.00	2.99	-2.32	1.52	1.40	0.20
Mallard	-1.26	-0.63	-0.60	4.96	1.00	-1.60	-0.91	-0.22	1.27
Mallard	-0.3	-0.58	-0.73	9.39	5.59	3.20	2.18	3.26	0.67
Northern Cardinal	-0.56	-0.58	-0.70	0.24	-0.09	0.76	0.01	-0.49	-1.04
Northern Cardinal	-1.33	-0.93	-1.29	3.68	7.89	7.58	0.53	-2.01	0.26
Northern Flicker	-0.74	-0.89	-1.19	-1.24	0.18	-0.19	0.15	0.10	0.99
Piping Plover	-0.66	-0.91	-1.00	-0.82	-0.86	-0.66	-2.10	0.43	-0.46
Purple Martin	-0.42	-0.49	-0.29	-0.46	-0.19	1.21	-0.91	0.67	1.65
Red-bellied Woodpecker	-1.03	-0.63	-0.77	3.50	-0.07	-2.72	0.13	1.25	1.46
Red-bellied Woodpecker	-0.32	-0.61	-0.90	-0.16	-1.26	-1.49	-0.44	-0.18	-0.89

Appendix B, continued.

Red-winged Blackbird	-0.59	0.55	-0.16	5.94	1.31	0.64	3.36	-0.04	0.32
Ring-billed Gull	-0.85	-0.77	0.22	-4.71	0.48	-4.10	-4.55	-5.48	-10.33
Rose-breasted Grosbeak	-0.2	0.05	0.34	0.91	0.68	-0.07	0.88	-0.23	2.54
Sanderling	-1.21	-0.85	-0.39	-0.60	-0.77	-1.33	-3.78	-1.27	6.03
Snowy Egret	-0.01	0.28	0.92	-2.08	-0.22	-0.13	0.42	1.66	2.02
Thrush sp.	-0.19	-0.63	-0.83	2.81	-0.34	-0.87	5.50	17.17	-0.21
Wood Duck	-1.09	-0.70	-0.50	3.30	0.40	-2.42	2.10	2.79	1.38
Wood Duck	-0.82	0.25	0.06	4.16	-0.22	-1.10	-1.64	-0.59	-0.95
Yellow-bellied Sapsucker	-0.39	-0.10	-1.05	1.68	-0.43	-0.18	1.06	-0.49	-0.62

VITA

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